IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Lorens et al.
Application No. 10/696,909
Filed: October 29, 2003
Confirmation No. 9257
For: MODULATORS OF ANGIOGENESIS AND TUMORIGENESIS
Examiner: Peter J. Reddig
Art Unit: 1642
Attorney Reference No. 7946-79836-01

FILED VIA EFS

FILED VIA EFS
COMMISSIONER FOR PATENTS

APPLICANTS' APPEAL BRIEF

This is an appeal brief filed under 37 C.F.R. § 41.37. A Notice of Appeal was received by the United States Patent and Trademark Office on January 3, 2011, making the Appeal Brief due on or before **March 3, 2011**. In accordance with 37 C.F.R. § 41.20(b)(2), this Appeal Brief is being filed together with the required small entity fee of $270. The Commissioner is hereby authorized to charge any deficiency in the required fee or to credit any overpayment to Deposit Account No. 02-4550.

I. REAL PARTY IN INTEREST

The real party in interest is Rigel Pharmaceuticals, Inc., the assignee of record of the present application (recorded at Reel 015425, Frames 0299-0303, December 3, 2004).

II. RELATED APPEALS AND INTERFERENCES

This application was previously the subject of Appeal No. 2009-011194, decided by the Board of Patent Appeals and Interferences (BPAI) on March 16, 2010. A copy of the Decision is submitted herewith. There are no pending related proceedings.

III. STATUS OF CLAIMS

Claims 1, 14-18, 27, 41-44, 54, and 55 are pending. Claims 2-13, 19-26, 28-40, 45-53, and 56-63 have been canceled. Claims 1, 14-18, 27, 41-44, 54, and 55 have been rejected, and are appealed. The pending claims are included in the attached Claims Appendix.

IV. STATUS OF AMENDMENTS

A response was filed with the Notice of Appeal on January 3, 2011, in reply to the Final Office Action of November 5, 2010. No claim amendments were included in this response. The claims were amended in the Amendment and Response to Non-final Office action submitted on August 17, 2010. These amendments were entered, as stated in the Final Office action dated November 5, 2010.

V. SUMMARY OF CLAIMED SUBJECT MATTER

The present invention stems from the discovery that Axl kinase (*e.g.*, SEQ ID NO: 4) is involved in angiogenesis. This discovery was made through Applicants' demonstration that treatment of human primary endothelial cells with Axl RNAi inhibited haptotaxis and tube formation of the endothelial cells, thus indicating Axl's role in angiogenesis. As embodied by independent claim 1, the invention at issue relates to a method for identifying a compound that inhibits angiogenesis by "assaying *in vitro* kinase activity of an Axl polypeptide comprising an amino acid sequence with greater than about 95% identity to full length SEQ ID NO: 4 in the

presence of the compound…; performing a cell-based assay in an endothelial cell comprising said Axl polypeptide in the presence of the compound, which assay produces an angiogenesis phenotype selected from the group consisting of αvβ3 expression, tube formation, and haptotaxis…; and identifying a compound that inhibits the *in vitro* kinase activity of the Axl polypeptide and that inhibits the angiogenesis phenotype in the cell-based assay, wherein inhibition of the *in vitro* kinase activity of the Axl polypeptide in the presence of the compound and inhibition of the angiogenesis phenotype in the cell-based assay in the presence of the compound identifies the compound as a compound that inhibits angiogenesis." See, *e.g.,* specification at page 8, line 15 to page 9, line 8; page 9, lines 32-34; page 30, lines 4-29; and page 31, lines 23-31; original claim 1.

As embodied by independent claim 27, the invention at issue also relates to a method for identifying a compound that inhibits angiogenesis by "contacting the compound with an endothelial cell that expresses a recombinant Axl polypeptide comprising an amino acid sequence with greater than about 95% identity to full length SEQ ID NO: 4…; performing a cell-based assay, which assay produces an angiogenesis phenotype selected from the group consisting of αvβ3 expression, tube formation, and haptotaxis in said endothelial cell…; and identifying a compound that inhibits the angiogenesis phenotype in the cell-based assay, wherein inhibition of the angiogenesis phenotype in the cell-based assay in the presence of the compound identifies the compound as a compound that inhibits angiogenesis." See, *e.g.,* specification at page 9, lines 32-34; page 31, line 23 to page 33, line 12.

VI. GROUNDS OF REJECTION FOR REVIEW

Claims 1, 14-18, 27, 41-44, 54, and 55 are rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Mor (U.S. Pat. App. Publication No. 2003/0157573) in view of Klinghoffer *et al.* (U.S. Pat. App. Publication No. 2004/0077574), further in view of O'Donnell *et al.* (*Am. J. Pathol.* 154:1171-1180, 1999), and further in view of Varner and Cheresh (*Curr. Opin. Cell Biol.* 8:724-730, 1996). This is the only rejection pending in the subject application.

VII. ARGUMENT

Claims 1, 14-18, 27, 41-44, 54, and 55 are rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Mor (U.S. Pat. App. Publication No. 2003/0157573) in view of Klinghoffer *et al.* (U.S. Pat. App. Publication No. 2004/0077574), further in view of O'Donnell *et al.* (*Am. J. Pathol.* 154:1171-1180, 1999), and further in view of Varner and Cheresh (*Curr. Opin. Cell Biol.* 8:724-730, 1996). Claims 14-18, 41-44, 54, and 55 each depend from claim 1 and/or 27. The rejection of claims 1, 14-18, 27, 41-44, 54, and 55 is argued as a group in this Appeal Brief.

The analysis for determining obviousness under 35 U.S.C. § 103(a), as articulated in *Graham v. John Deere Co.* 383 U.S. 1 (1966), requires 1) determining the scope and content of the prior art; 2) ascertaining the differences between the prior art and the claims at issue; and 3) resolving the level of ordinary skill in the pertinent art. *Graham*, 383 U.S. at 7. In particular, ascertaining the differences between the prior art and the claims requires that both the claims and the prior art be read as a whole (M.P.E.P. § 2141.02; *In re Langer*, 465 F.2d 896, 899, 175 USPQ 169, 171 (CCPA 1972); *W.L. Gore & Associates v. Garlock, Inc.*, 721 F.2d 1540, 1551, 220 USPQ 303, 311 (Fed. Cir. 1983), *cert. denied*, 469 U.S. 851 (1984)). "All of the disclosures in a reference must be evaluated for what they fairly teach one of ordinary skill in the art…[W]hen 'all of the disclosures in a reference' are considered, the overall suggestion to emerge from the prior art reference may be contrary to that which might appear from an isolated portion of the reference." *In re Langer*, 465 F. 2d at 899, 175 USPQ at 171.

To establish a *prima facie* case of obviousness, the Office must establish that (1) there is some suggestion or motivation to combine the references, either in the references or in common general knowledge of one of skill in the art (MPEP § 2143.01); and (2) there is a reasonable expectation of success (MPEP § 2143.02). In addition, the Office must show that the references teach or suggest all claim limitations. "When determining whether a claim is obvious, an Examiner must make 'a searching comparison of the claimed invention – *including all its limitations* – with the teaching of the prior art.' Thus, 'obviousness requires a suggestion of all limitations in a claim.'" *Ex parte Mumper* BPAI, Appeal No. 2008-2332, June 27, 2008.

The Office asserts that Mor specifically teaches that compounds identified in the assays disclosed in that reference could be used as anti-angiogenic drugs, that O'Donnell *et al.* show that Axl is expressed in endothelial cells and is involved in their viability and survival, and that Varner and Cheresh teach that $\alpha v\beta 3$ is important in endothelial cell survival (Office action of November 5, 2010, page 6, second full paragraph). The Office asserts that, "given the art teaches that these are important aspects of angiogenesis by endothelial cells, it would have been obvious to one of skill in the art to assay these function in an effort to identify an angiogenesis inhibitor in addition to assaying a test compound's effect on Axl activity, given that they both have a role in endothelial cell function and angiogenesis" (Office action of November 5, 2010, page 6, second full paragraph).

The Office also states that Klinghoffer *et al.* disclose that siRNA is an advantageous inhibitory molecule. The Office asserts that it would have been obvious to one of skill in the art to "combine the teachings of Mor and Klinghoffer *et al.* and use RNAi molecules in the screening methods of Mor…to identify the most effective inhibitory molecule…" (Office action of November 5, 2010, page 4, first full paragraph).

Applicants assert that, when read as a whole, neither Mor nor O'Donnell *et al.* (nor any of the references used to support this rejection) teach or suggest that Axl plays a role in angiogenesis, and therefore one of skill in the art would not be motivated to utilize the method of Mor to identify a compound that inhibits angiogenesis (for example by assaying tube formation, haptotaxis, or $\alpha v\beta 3$ expression). Furthermore, one of skill in the art would not have had a reasonable expectation of success in combining these references to arrive at Applicants' claimed method. Thus, the Office has not met the burden needed to support a *prima facie* case of obviousness for claims 1, 14-18, 27, 41-44, 54, and 55 on the basis of the cited references.

A. No Motivation to Combine the References

"Obviousness can be established by combining or modifying the teachings of the prior art to produce the claimed invention where there is some teaching, suggestion, or motivation to do so." M.P.E.P. § 2143.01. As discussed above, the Office asserts that one of skill in the art

would be motivated to combine the cited references to identify a compound that inhibits angiogenesis.

The rationale to combine the prior art "may be expressly or impliedly contained in the prior art or it may be reasoned from knowledge generally available to one of ordinary skill in the art, established scientific principles, or legal precedent established by prior case law." M.P.E.P. § 2144. In this case, there is no rationale to combine the references, particularly when the references are read as a whole, as required by case law and the M.P.E.P (discussed above).

The focus of Mor is on identification of genes which have changes in expression in renal pathologies, and use of these sequences for screening for treatment modalities for fibrosis in general, and specifically renal fibrosis and glomerulosclerosis (paragraph [0002], Field of the Invention). Mor discloses a method for identifying compounds for treating renal disease (specifically diabetic nephropathy), fibrosis (particularly renal fibrosis) and glomerulosclerosis (see, *e.g.*, paragraphs [0002], [0032-0033], [0036], [0041], and [0096]). Mor discloses that Axl expression is increased in fibrotic kidney in humans and in animal models of renal fibrosis (*e.g.*, paragraphs [0240] and [0249-0250]), and suggests that therapeutic approaches aimed at Axl would be beneficial for both chronic and acute renal failure (paragraph [0251]). Thus, taken as a whole, Mor is directed to the role of Axl in renal pathology, and more specifically, renal fibrosis.

In addition, the assays disclosed in Mor that measure the effect of a test compound on a cellular phenotype assess cell survival, cellular differentiation, or cell proliferation (paragraph [0059]). Specific assays include proliferation of mesangial cells, renal fibroblasts, or renal tubular cells, collagen deposition in the extracellular matrix of renal fibroblasts, and transdifferentiation of renal tubular cells to myofibroblasts (paragraph [0069]). Thus the focus of Mor is also on the possible role of Axl in cell proliferation or survival.

The Office points to paragraph [0090] of Mor as teaching that compounds identified in the disclosed assays for inhibition of Axl may be used as anti-angiogenic drugs. Mor specifically states that the identified compounds "may also be used as anti-angiogenic drugs for the treatment of cancer and other conditions where preventing or reducing *proliferation* of endothelial cells is

desired" (paragraph [0090], emphasis added). Applicants emphasize that this single sentence is the only mention of angiogenesis in the entirety of the Mor reference (the specification is 20 pages long). Furthermore, Mor states that a compound that inhibits Axl may prevent or reduce *proliferation* of endothelial cells. One of skill in the art would recognize that a compound may reduce proliferation of endothelial cells without being anti-angiogenic (see, *e.g.,* Frater-Schroder *et al., Proc. Natl. Acad. Sci. USA* 84:5277-5281; page 5277, col. 2, first paragraph; discussed in detail in Section B, below). In addition, Mor analyzed the expression of Axl in the rat and found that Axl is widely expressed in many tissues and cell types, including intestinal tract, skin, salivary gland, heart, prostate, liver portal tract (fibroblasts and histiocytes/macrophages), spleen, lymph node, thymus, lung (macrophages and/or lymphocytes), liver sinusoidal cells (endothelial, stellate, and Kuppfer cells), testis (Sertoli cells and germ cells), and brain (glial cells) (paragraphs [0252-0257]). This pattern of expression in a wide variety of tissues and cell types would not suggest to one of skill in the art that Axl is involved in angiogenesis. Given the overall teaching of Mor that Axl is involved in renal pathology (particularly fibrosis) and is widely expressed, one of skill in the art would not consider that Mor suggests that Axl is involved in angiogenesis. At most, one of skill in the art would be led to conclude that Axl may be involved in cell proliferation and that the assays described in Mor may be used to identify inhibitors of cell proliferation.

The Office relies on O'Donnell *et al.* to provide motivation to measure tube formation in the assay of Mor (Office action of November 5, 2010, page 4, second full paragraph) and motivation to identify anti-angiogenic compounds because Axl is expressed in endothelial cells and is involved in their viability and survival (Office action of November 5, 2010, page 6, second full paragraph).

O'Donnell *et al.* disclose that Axl is expressed in synovial tissue from patients with rheumatoid arthritis, particularly in endothelial cells in subsynovial capillaries, smooth muscle cells in arterioles and veins, and synovial lining cells (*e.g.,* page 1173, col. 2 to page 1174, col. 1). O'Donnell *et al.* also disclose that the Axl ligand Gas6 increases endothelial cell survival and/or reduces endothelial cell apoptosis in response to growth factor depletion or treatment with tumor necrosis factor α (abstract; page 1174, col. 2 to page 1176, col. 1). O'Donnell *et al.* state

that there is a *possibility* that Axl is involved in vascular structure and function (page 1176, col. 2, second full paragraph). However, O'Donnell *et al.* also specifically state that "the major role of Axl-Gas6 interaction may therefore be in *survival* of the vasculature under conditions of cellular stress or injury" and "may also promote *survival* of activated endothelial cells, and perhaps other Axl-positive cells, within the hostile environment of the inflamed rheumatoid joint" (page 1179, col. 1, emphasis added). Finally, O'Donnell *et al.* suggest that this "*survival* mechanism normally involved in tissue homeostasis could also contribute to *maintenance* of a pathological vasculature" (page 1179, col. 1, emphasis added). Thus, when read as a whole O'Donnell *et al.* is clearly directed to the role of Axl and its ligand Gas6 in survival or viability of endothelial cells. While endothelial cells are required for angiogenesis, signals that promote the survival or proliferation of endothelial cells do not equate to angiogenic signals (discussed further in Section B, below).

The Office states that "O'Donnell *et al.* teaches that Axl may be involved in tube formation during [] angiogenesis" (Office action of November 5, 2010, page 4, second full paragraph). O'Donnell *et al.* actually state that "homophilic binding between the extracellular domains of Axl has been demonstrated. This suggests a role in cell adhesion which could be relevant to tube formation in angiogenesis. Vascular smooth muscle cell expression has been previously noted in the rat and may suggest involvement of Axl in *some other aspect* of vascular function" (p. 1176, last 7 lines; emphasis added). O'Donnell *et al.* further note that Gas6 is a "promiscuous ligand" for the Axl subfamily (which includes Axl, Sky, and Mer tyrosine kinases) and that "Gas6 has been shown to protect a number of Axl-positive cells from stimuli that induce apoptosis" (page 1178, col. 2, second full paragraph). Other "nonmitogenic" effects of Gas6 (such as chemotaxis) may be due to the "promiscuous" effects of Gas6 and not specific to Axl. Thus the suggestion that Axl is involved in cell adhesion, which could be relevant to tube formation, is highly speculative, given that this effect could be mediate by Gas6 through one of its other receptors (such as Sky or Mer).

The disclosure of O'Donnell *et al.* is highly similar to that of Healy *et al.* (*Am. J. Physiol. Lung Cell Metabol.* 280:L1273-L1281, 2001), which was previously cited by the Office in a rejection under 35 U.S.C. § 103(a) in combination with Varner and Cheresh and Klinghoffer *et*

al. (*e.g.,* Office action dated June 23, 2008). Similar to O'Donnell *et al.*, Healy *et al.* disclose that Gas6 increased cell number and decreased apoptosis of endothelial cells which express Axl polypeptide (*e.g.,* Healy *et al.,* page L1276, col. 2, last paragraph; page L1277, col. 2; and page 1278, col. 2). Healy *et al.* also disclose that apoptosis plays a role in vascular remodeling associated with tumor angiogenesis (page 1280, last paragraph). However, in the Decision of the BPAI in the previous appeal in this application, the BPAI found that Healy *et al.* did not provide sufficient motivation for one of skill in the art to assay an angiogenesis marker such as $\alpha v\beta 3$ expression in endothelial cells (BPAI Decision of Appeal 2009-011194, March 16, 2010, page 17, last paragraph; attached). Similarly, although O'Donnell *et al.* includes speculative statements to the effect that Axl *could perhaps* play a role in some cellular events that are associated with a number of processes (including, but not limited to angiogenesis), this does not provide sufficient motivation for one of skill in the art to consider that an Axl inhibitor would be an inhibitor of angiogenesis or to assay angiogenesis phenotypes selected from $\alpha v\beta 3$ expression, tube formation, and haptotaxis in a cell-based assay, as in Applicants' claims.

Based on the focus of Mor on using an assay for inhibitors of Axl to identify compounds for use in treating renal fibrosis and glomerulosclerosis, conditions which are not associated with angiogenesis, and the focus of O'Donnell *et al.* on the role Gas6-Axl in endothelial proliferation and/or survival, one of skill in the art would not have been motivated at the time of Applicants' filing to combine the disclosures of Mor and O'Donnell *et al.* to develop an assay for inhibitors of Axl that would identify an inhibitor of angiogenesis. Although O'Donnell *et al.* speculate as to the possible role of Axl and its ligand Gas6 in cellular functions such as cellular adhesion and chemotaxis (which in some instances are involved in angiogenesis), one of skill in the art would not read this reference as whole as suggesting that inhibitors of Axl will be inhibitors of angiogenesis. Cell adhesion and chemotaxis are cellular functions that occur during angiogenesis, but they also occur in many other processes, such as inflammation and tumor metastasis. Thus, particularly given the focus of Mor on fibrosis and glomerulosclerosis, one of skill in the art would not expect Axl to be involved in angiogenesis based on the speculative statements regarding cell adhesion and chemotaxis found in O'Donnell *et al.* Similarly, endothelial cell survival or proliferation are cellular functions that occur during angiogenesis, but

also occur during other processes, such as maintenance of vascular structure under conditions of cellular stress or injury (as pointed out in O'Donnell *et al.* at page 1179, col. 1).

Varner and Cheresh describe the role of αvβ3 integrin in the process of angiogenesis (page 726, right column) and disclose that an αvβ3 antagonist inhibits angiogenesis (page 726-727). However, this reference does not teach or suggest a role for Axl polypeptide in angiogenesis. Without a reasonable expectation that Axl is involved in angiogenesis (which is *not* provided by Mor or O'Donnell *et al.,* alone or in combination), one of skill in the art would not be motivated to measure αvβ3 expression in the assay of Mor. Therefore, the combination of Varner and Cheresh with Mor and O'Donnell *et al.* does not provide a motivation for one of skill in the art to measure αvβ3 expression in the assay of Mor.

Finally, Klinghoffer *et al.* merely disclose siRNAs and their use as therapeutics for a wide range of diseases. Like Varner and Cheresh, Klinghoffer *et al.* does not teach or suggest a role for Axl polypeptide in angiogenesis. Thus, there is no motivation for one of skill in the art to utilize siRNAs in the assay of Mor to identify inhibitors of angiogenesis, even in combination with the disclosure of O'Donnell *et al.* and/or Varner and Cheresh.

B. No Reasonable Expectation of Success

An additional element of a *prima facie* case of obviousness is that the prior art must support a reasonable expectation of success for achieving the invention. "The prior art can be modified or combined to reject claims as *prima facie* obvious as long as there is a *reasonable expectation* of success." M.P.E.P. § 2143.02 (emphasis added). The references cited in the rejection of claims 1, 14-18, 27, 41-44, 54, and 55 under 35 U.S.C. § 103(a) do not support a reasonable expectation of success for achieving Applicants' claimed invention, in light of the knowledge of one of skill in the art. Therefore, the Office has not met this requirement for establishing *prima facie* obviousness.

As discussed above in Section A, both Mor and O'Donnell *et al.* are strongly focused on cell proliferation and survival with respect to the cellular function of Axl (*e.g.,* Mor, paragraphs [0036], [0059], [0069], [0161-1063], and [0241]; O'Donnell *et al.,* page 1172, col. 1, first

paragraph; page 1174, col. 2, last paragraph to page 1176, col. 2, top; page 1179, col. 1). Neither Varner and Cheresh nor Klinghoffer *et al.* provide any information on the potential cellular function of Axl. Therefore, one of skill in the art would not have had a reasonable expectation of success for achieving the claimed methods for identifying a compound that inhibits angiogenesis by combining the teachings of these references, particularly when each reference is read as a whole.

It is not predictable that a compound that inhibits cell proliferation, even endothelial cell proliferation, is also a compound that also inhibits angiogenesis. For example, Frater-Schroder *et al.* (*Proc. Natl. Acad. Sci. USA* 84:5277-5281, 1987; cited in the Office action of December 12, 2007) discloses that tumor necrosis factor α (TNF-α) inhibits proliferation of endothelial cells in culture, but stimulates neovascularization in an *in vivo* assay (*e.g.,* page 5277, col. 2, first paragraph; page 5278, col. 1-2; page 5279, col. 1, first full paragraph). This property of inhibition of endothelial cell proliferation in culture, but stimulation of angiogenesis *in vivo,* is not unique to TNF-α. Frater-Schroder *et al.* point out that transforming growth factor β (TGF-β) exhibits these same characteristics (*e.g.,* page 5279, col. 2, third full paragraph and page 5280, col. 1-2).

Mor states that inhibitors of Axl identified in the assays disclosed in that reference could be used for treatment of conditions "where preventing or reducing proliferation of endothelial cells is desired" (Mor, paragraph [0090]) and merely makes the conclusory statement that antagonists of Axl could be anti-angiogenic drugs. There is no disclosure that Axl is involved in any processes related to angiogenesis. Mor only provides data showing that Axl expression is increased in tubular epithelial cells in fibrotic kidney regions and suggests that Axl is involved in cell proliferation in these regions (Mor, paragraphs [0239-0240]). As discussed above, it is not predictable that an inhibitor of cell proliferation is an inhibitor of angiogenesis. Therefore, one of skill in the art would not have had a reasonable expectation that an inhibitor of Axl would be an inhibitor of angiogenesis at the time of filing of the present application.

The data presented by O'Donnell *et al.* is entirely focused on the expression of Axl in synovial tissue and the ability of Axl's ligand Gas6 to protect human umbilical vein endothelial

cells from apoptosis (O'Donnell *et al.,* pages 1174-1176). O'Donnell *et al.* provide two statements regarding the ability of Gas6 to promote cell adhesion between cells expressing Axl and suggest that this *could* play a role in tube formation or chemotaxis (page 1176, col. 2, second full paragraph). However, these statements are entirely speculative and are not supported by any evidence in O'Donnell *et al.* (or the other references cited by the Office). O'Donnell *et al.* also note that Axl was previously found to be expressed in vascular smooth muscle cells in the rat and "may suggest involvement of Axl in *some other aspect* of vascular function" rather than angiogenesis (O'Donnell *et al.*, page 1176, last 4 lines).

When read as a whole, both Mor and O'Donnell *et al.* only disclose that Axl plays a role in cell proliferation and survival. As it is not predictable that a compound that inhibits endothelial cell proliferation also inhibits angiogenesis, one of skill in the art would not have had a reasonable expectation of success that the assays of Mor could be used to identify a compound that inhibits angiogenesis, for example, utilizing tube formation or chemotaxis, which are mentioned only in passing by O'Donnell *et al.*

Furthermore, Varner and Cheresh disclose the potential role of the integrin $\alpha v\beta 3$ in tumor cell proliferation and survival (*e.g.,* Varner and Cheresh, page 725, col. 2, third full paragraph and page 727, col. 1-2) and tumor angiogenesis (*e.g.,* Varner and Cheresh, page 726, col. 2, second full paragraph to page 727, col. 1, second paragraph). Klinghoffer *et al.* discuss use of siRNA for modulating biological signal transduction (Klinghoffer *et al.,* paragraph [0028]). Neither Varner and Cheresh nor Klinghoffer *et al.* disclose any potential cellular function for Axl, let alone a potential role in angiogenesis. Thus, the combination of these two references with Mor and O'Donnell *et al.* would not have provided one of skill in the art with a reasonable expectation of success for achieving the claimed methods for identifying a compound that inhibits angiogenesis.

C. Obviousness under the Post-KSR v. Teleflex Guidelines

Following the Supreme Court decision in *KSR International Co. v. Teleflex Inc.*, 550 U.S. 398, 82 USPQ2d 1385 (2007), the Office published Examination Guidelines which included exemplary rationales that may support a conclusion of obviousness (72 Fed. Reg. 57526-57535,

October 10, 2007; "Guidelines"). These rationales have subsequently been incorporated in the M.P.E.P. at § 2143. The rationales provided in the Guidelines and M.P.E.P. § 2143 that may be relevant to the pending appeal include "(A) combining prior art elements according to known methods to yield *predictable results*; (B) simple substitution of one known element for another to obtain *predictable results*; ... (E) 'obvious to try' – choosing from a finite number of identified *predictable solutions, with a reasonable expectation of success*; ... (G) some teaching, suggestion, or motivation in the prior art that would have led one of ordinary skill to modify the prior art reference or to combine prior art reference teachings to arrive at the claimed invention" (M.P.E.P. § 2143, emphasis added). Applicants have based their arguments above on the last rationale (G), as this appears to be the rationale set forth in the Office action of November 5, 2010, and the Advisory Action of January 27, 2011.

Applicants assert that the arguments set forth above demonstrate that the Office has not made a *prima facie* case of obviousness, even if one of the other rationales set forth in the Guidelines and M.P.E.P. § 2143 were applied. Each of the rationales set forth as (A), (B), and (E) requires that the combination of elements yield **predictable results** or a **reasonable expectation of success**. As discussed in part B above, prior to the filing of this application, one of skill in the art would *not* have had any reasonable expectation of success in achieving Applicants' claimed methods based on the cited references. Therefore, even if one of the other rationales is applied, the Office has not met its burden to demonstrate a *prima facie* case of obviousness in rejecting claims 1, 14-18, 27, 41-44, 54, and 55.

D. Conclusion

Applicants have shown that the Office has not established a *prima facie* case of obviousness, because one of skill in the art would not have been motivated to combine the cited references nor have a reasonable expectation of success to arrive at Applicants' claims. Therefore, the claims are not obvious in light of the cited references.

In view of the above remarks, Applicants believe that they have overcome the rejection of claims 1, 14-18, 27, 41-44, 54, and 55 under 35 U.S.C. § 103(a). Applicants respectfully request that the rejection of claims 1, 14-18, 27, 41-44, 54, and 55 be withdrawn.

Respectfully submitted,

KLARQUIST SPARKMAN, LLP

By /Susan W. Graf/
Susan W. Graf, Ph.D.
Registration No. 60,432

One World Trade Center, Suite 1600
121 S.W. Salmon Street
Portland, Oregon 97204
Telephone: (503) 595-5300
Facsimile: (503) 595-5301

cc: Docketing

VIII. CLAIMS APPENDIX

1. (Rejected) A method for identifying a compound that inhibits angiogenesis, the method comprising:

assaying *in vitro* kinase activity of an Axl polypeptide comprising an amino acid sequence with greater than about 95% identity to full length SEQ ID NO: 4 in the presence of the compound, wherein the Axl polypeptide has kinase activity in the absence of said compound;

performing a cell-based assay in an endothelial cell comprising said Axl polypeptide in the presence of the compound, which assay produces an angiogenesis phenotype selected from the group consisting of $\alpha v\beta 3$ expression, tube formation, and haptotaxis in said endothelial cell in the absence of the compound; and

identifying a compound that inhibits the *in vitro* kinase activity of the Axl polypeptide and that inhibits the angiogenesis phenotype in the cell-based assay,

wherein inhibition of the *in vitro* kinase activity of the Axl polypeptide in the presence of the compound and inhibition of the angiogenesis phenotype in the cell-based assay in the presence of the compound identifies the compound as a compound that inhibits angiogenesis.

2-13. (Canceled)

14. (Rejected) The method of claim 1, wherein the polypeptide is recombinant.

15. (Rejected) The method of claim 1, wherein the compound is an antibody.

16. (Rejected) The method of claim 1, wherein the compound is an antisense molecule.

17. (Rejected) The method of claim 1, wherein the compound is an RNAi molecule.

18. (Rejected) The method of claim 1, wherein the compound is a small organic molecule.

19-26. (Canceled)

27. (Rejected) An *in vitro* method for identifying a compound that inhibits angiogenesis, the method comprising:

contacting the compound with an endothelial cell that expresses a recombinant Axl polypeptide comprising an amino acid sequence with greater than about 95% identity to full length SEQ ID NO: 4, wherein the Axl polypeptide has kinase activity in the absence of said compound;

performing a cell-based assay, which assay produces an angiogenesis phenotype selected from the group consisting of $\alpha v\beta 3$ expression, tube formation, and haptotaxis in said endothelial cell in the absence of the compound; and

identifying a compound that inhibits the angiogenesis phenotype in the cell-based assay,

wherein inhibition of the angiogenesis phenotype in the cell-based assay in the presence of the compound identifies the compound as a compound that inhibits angiogenesis.

28-40. (Canceled)

41. (Rejected) The method of claim 27, wherein the compound is an antibody.

42. (Rejected) The method of claim 27, wherein the compound is an antisense molecule.

43. (Rejected) The method of claim 27, wherein the compound is an RNAi molecule.

44. (Rejected) The method of claim 27, wherein the compound is a small organic molecule.

45-53. (Canceled)

54. (Rejected) The method of claim 1 or 27, wherein the Axl polypeptide comprises SEQ ID NO: 4.

55. (Rejected) The method of claim 1, wherein inhibition of the angiogenesis phenotype in the cell-based assay is caused by down regulation of expression of the Axl polypeptide.

56-63. (Canceled)

IX. EVIDENCE APPENDIX

Frater-Schroder *et al., Proc. Natl. Acad. Sci. USA* 84:5277-5261, 1987 (cited by the Office on December 12, 2007)

Healy *et al., Am. J. Physiol. Lung Cell Metabol.* 280:L1273-L1281, 2001 (first cited by the Office on August 23, 2006)

Klinghoffer *et al.,* U.S. Pat. App. Publ. No. 2004/0077574 (first cited by the Office on June 23, 2008)

Mor, U.S. Pat. App. Publ. No. 2003/0157573 (first cited by the Office on May 27, 2010)

O'Donnell *et al., Am. J. Pathol.* 154:1171-1180, 1999 (cited by Applicants in the Information Disclosure Statement submitted December 1, 2006; first cited by the Office on May 27, 2010)

Varner and Cheresh, *Curr. Opin. Cell Biol.* 8:724-730. 1996 (first cited by the Office on August 23, 2006)

X. RELATED PROCEEDINGS APPENDIX

This application was previously the subject of Appeal No. 2009-011194, decided by the Board of Patent Appeals and Interferences (BPAI) on March 16, 2010. A copy of the BPAI Decision is submitted herewith. There are no pending related proceedings.

Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 5277–5281, August 1987
Cell Biology

Tumor necrosis factor type α, a potent inhibitor of endothelial cell growth *in vitro*, is angiogenic *in vivo*

(fibroblast growth factor/bovine aortic endothelial cells/brain capillary endothelial cells/smooth muscle cells/rabbit cornea)

MARIJKE FRÄTER-SCHRÖDER*, WERNER RISAU†, RUPERT HALLMANN†, PETER GAUTSCHI*, AND PETER BÖHLEN‡

*Department of Biochemistry, University of Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland; and *Max-Planck-Institute for Developmental Biology, D-7400 Tübingen 1, Federal Republic of Germany

Communicated by Robert W. Holley, April 20, 1987 (received for review February 23, 1987)

ABSTRACT **Tumor necrosis factor type α (TNF-α) inhibits endothelial cell proliferation *in vitro*. Basal cell growth (in the absence of exogenously added growth factor) and fibroblast growth factor (FGF)-stimulated cell proliferation are inhibited in a dose-dependent manner from 0.1 to 10 ng/ml with half-maximal inhibition occurring at 0.5–1.0 ng of TNF-α per ml. Bovine aortic and brain capillary endothelial and smooth muscle cells are similarly affected. TNF-α is a noncompetitive antagonist of FGF-stimulated cell proliferation. Its action on endothelial cells is reversible and noncytotoxic. Surprisingly, TNF-α does not seem to inhibit endothelial cell proliferation *in vivo*. In the rabbit cornea, even a high dose of TNF-α (10 μg) does not suppress angiogenesis induced by basic FGF. On the contrary, in this model system TNF-α stimulates neovascularization. The inflammatory response that is seen in the cornea after TNF-α implantation suggests that the angiogenic properties of this agent may be a consequence of leukocyte infiltration.**

Tumor necrosis factor type α (TNF-α) is a polypeptide originally identified in the serum of mice infected with bacillus Calmette–Guérin and then treated with endotoxin (1). This protein was later isolated from macrophages (2) and its structure was determined by cDNA cloning (3). It is identical to cachectin (2) and structurally (4) and biologically (5) related to the lymphocyte product lymphotoxin (TNF-β). TNF-α causes hemorrhagic necrosis and complete regression of certain transplanted tumors in mice (1), induces wasting (cachexia) and a lethal state of shock (6), and inhibits metastasis formation in animals (7). A variety of *in vitro* effects have been reported: TNF-α is cytostatic or cytolytic for several human or murine carcinoma, melanoma, and sarcoma cell lines and also for virally transformed 3T3 cells (8, 9). However, TNF-α is not cytotoxic or growth inhibitory for various normal cells (1, 6). It can even stimulate the proliferation of some cell types (9, 10). Furthermore, TNF-α suppresses lipoprotein lipase activity in adipocytes (11) and collagen and proteoglycan synthesis in osteoclasts (12) and cartilage (13), respectively. It has been shown to stimulate the formation of prostaglandin E_2 (14, 15), collagenase (15), interleukin 1 (14, 16), interferons (17, 18), and granulocyte/macrophage colony-stimulating factor (GM-CSF) (19) in fibroblasts, macrophages, or synovial cells. TNF-α is also antiviral for a number of cell types (20, 21). Finally, TNF-α exhibits a variety of activities toward endothelial cells, including the stimulation of procoagulant activity (22, 23), GM-CSF (19, 24), interleukin 1 (16), cell-surface antigen expression (25, 26) and the inhibition of proteoglycan synthesis (13) and cell growth (18, 27). Those observations suggest that the vascular endothelial system may be a target for TNF-α action *in vivo*.

The mechanism for TNF-α-induced tumor necrosis and regression is unknown. Recently observed inhibitory effects of TNF-α on endothelial cells raise the question whether TNF could affect tumor necrosis/regression, at least partially, through inhibition of endothelial cell proliferation *in vivo*—i.e., inhibition of tumor neovascularization. To investigate this hypothesis we studied the effect of TNF-α on endothelial cell proliferation *in vitro* and on angiogenesis *in vivo*. We report that TNF-α is a potent noncytotoxic growth inhibitor for endothelial cells in culture but enhances rather than blocks neovascularization.

MATERIALS AND METHODS

Recombinant human TNF-α (produced in *Escherichia coli*) was provided by Knoll GmbH (Ludwigshafen, Federal Republic of Germany). The purity of TNF-α was >99%, its specific activity (7.4×10^6 units/mg of protein) was tested in an L 929 cytotoxicity assay (without actinomycin D), the endotoxin level was 0.07 ng/mg of protein, and residual bacterial proteins were 50 ng/mg of protein. Basic and acidic fibroblast growth factors (bFGF and aFGF) were isolated from bovine pituitary and brain, respectively, as described (28, 29).

Cell Culture. Bovine aortic arch endothelial cells were prepared and cultured (passages 2–11) in Dulbecco's modified Eagle's medium (DMEM) with 10% calf serum (Hyclone, Logan, UT) in the presence of bFGF or aFGF as described (28–30). Bovine brain capillary endothelial cells were provided by D. Gospodarowicz (University of California, San Francisco) and cultured as described for aortic endothelial cells. Bovine smooth muscle cells were prepared from the aortic arch as described (31) and grown in the medium used for endothelial cells. Endothelial cells were identified by using fluorescently labeled acetylated low density lipoprotein (32) and smooth muscle cells were identified by their typical hill-and-valley morphology at confluence (31).

Growth-Inhibition Assay *in Vitro*. Cells were seeded in 35-mm plastic dishes (Falcon) at densities of 10,000–100,000 cells per dish, depending on cell type, and grown for 5–7 days in the presence of TNF-α alone or TNF-α and approximately maximally stimulating concentrations of bFGF (1 ng/ml) or aFGF (100 ng/ml). Unless otherwise stated, TNF-α and FGF were added immediately after plating of cells and again on day 2 of culture. At the end of the experiments, cells were trypsinized and counted in a Coulter particle counter. Indi-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: TNF-α, tumor necrosis factor type α; TNF-β, lymphotoxin; FGF, fibroblast growth factor(s); aFGF, acidic FGF; bFGF, basic FGF; GM-CSF, granulocyte/macrophage colony-stimulating factor; TGF-β, transforming growth factor type β.
‡To whom reprint requests should be addressed.

vidual values deviated no more than 10% from mean values. Variations between experiments, which were done at least twice, and between different cell types were sometimes >10% and were probably due to specific rates of basal cell growth, which differ strongly between the cell types. Further details are contained in the figure legends.

Determination of Cytotoxicity. *Long-term cytotoxicity.* Confluent endothelial cells [negative mycoplasma test (33), data not shown] cultured in DMEM/10% calf serum in 35-mm dishes were treated with various doses of TNF-α for 5 or 10 days. At the end of the incubation period the number of adherent cells was determined and compared to cell counts obtained with untreated cells.

Short-term cytotoxicity. Confluent aortic endothelial cells in 24 multiwell plates (Nunc) were labeled with ^{111}In as described (34, 35). Briefly, 20 μl of ^{111}indium chloride (50 mCi/ml; 1 Ci = 37 GBq; New England Nuclear) was added to 100 ml of 0.2 μM Tropolone (Serva, Heidelberg) in DMEM/10% calf serum. Cells were incubated with 500 μl of this solution for 15 min at 37°C and washed extensively. Under these conditions ≈5% of the label was incorporated into the cells. TNF-α (in 500 μl of culture medium) was added to the washed cells, and cells were incubated for 4 or 10 hr at 37°C. Aliquots of the medium were then counted in a γ-counter. Maximal ^{111}In release was determined in supernatants of cells lysed with 0.5% Triton X-100 in phosphate-buffered saline for 20 min at room temperature.

Angiogenesis Assay. Elvax (ethylene vinyl acetate) pellets (36) containing 50–500 ng of bFGF and/or 0.5–50 μg of TNF-α and a constant amount of rabbit serum albumin (to achieve 20% loading of the polymer) were prepared and implanted in the rabbit cornea and the response was evaluated as described (36, 37).

RESULTS

TNF-α inhibits the basal proliferation of bovine aortic and capillary endothelial cells cultured in serum-containing medium (Fig. 1). Inhibition was dose-dependent from 0.1 to 10 ng of TNF-α per ml with 50% inhibition occurring at ≈1 ng/ml (Fig. 1). The proliferation of those cells was also inhibited at similar TNF-α doses, when cell growth was stimulated by the addition of bFGF or aFGF (Fig. 2).

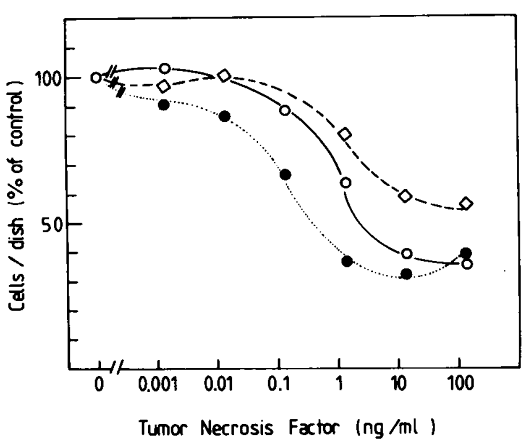


FIG. 1. Inhibition of basal (serum-stimulated) cell growth by TNF-α. Aortic endothelial cells (●), capillary endothelial cells (○), and smooth muscle cells (◇), all seeded at 100,000 cells per dish, were grown in the presence of various doses of TNF-α for 7 days. Cell growth is expressed as the percentage relative to that of untreated cells. Cell counts for untreated cultures were 750,000 and 620,000 cells per dish for endothelial and smooth muscle cells, respectively.

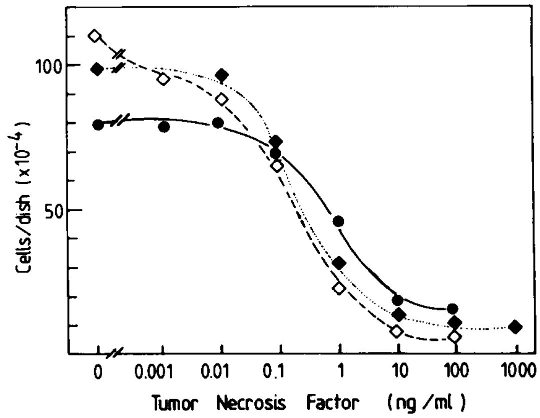


FIG. 2. Inhibition of FGF-stimulated endothelial cell growth by TNF-α. Cells were seeded at 20,000 cells per dish and grown for 5 days in the presence of various concentrations of TNF-α and maximally stimulating concentrations of either bFGF (1 ng/ml) or aFGF (100 ng/ml). In the absence of exogenous factors the aortic cells grew to a density of 60,000 cells per dish and the capillary cells grew to 160,000 cells per dish. ●, Capillary endothelial cells treated with TNF-α and bFGF; ◆, aortic endothelial cells treated with TNF-α and bFGF; ◇, aortic endothelial cells treated with TNF-α and aFGF.

Furthermore, TNF-α inhibited smooth muscle cell growth in the same dose range (Fig. 1).

TNF-α acts as a noncompetitive antagonist of FGF-stimulated endothelial cell growth. This conclusion is based on the observation that bFGF stimulated cell growth in an identical dose-dependent fashion (with very similar half-maximal stimulatory concentrations), regardless of whether TNF-α was added (Fig. 3). Furthermore, supramaximal doses of bFGF (e.g., 10 ng/ml, a 10-fold excess over the saturating concentration) were ineffective in overcoming the TNF-induced antiproliferative effect on cell growth.

Two experimental approaches did not show TNF-α cytotoxicity for bovine endothelial cells. In the long-term cytotoxicity assay (Table 1), the number of cells that remained attached to the culture dish (presumably the viable cells) was

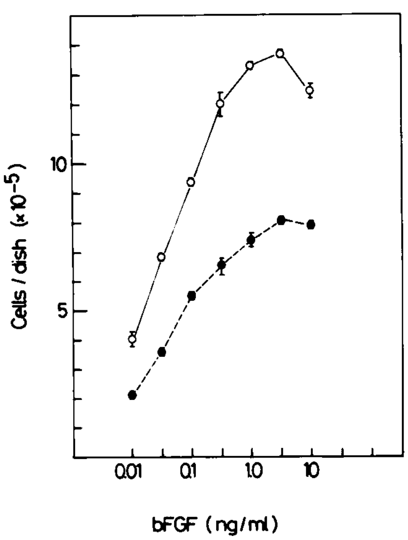


FIG. 3. Effect of bFGF on TNF-α-induced growth inhibition of aortic endothelial cells. Cells were grown with increasing bFGF concentrations in the absence of TNF-α (○) or with 1 ng of TNF-α per ml (●). Cell density at the time of seeding was 20,000 cells per 35-mm dish. Data are presented as means ± SD.

Table 1. Effect of 10-day exposure of endothelial cells to TNF-α on cell numbers

TNF-α, ng/ml	Adherent cells per dish, % of control
0	100 ± 15.3
1.4	70 ± 6.5
14	80 ± 4.0
140	77 ± 2.1

Values are means ± SD of triplicates.

consistently but only slightly (20–30%) lower in TNF-α-treated than in control cultures, regardless of the TNF-α dose. The apparently lower number of attached cells after TNF-α treatment is likely not to be due to a cytotoxic effect, because confluent endothelial cells continue to proliferate at a low rate (data not shown), whereas in TNF-α-treated cells this residual proliferation is suppressed. In a short-term cytotoxicity assay, TNF-α did not cause an increased leakage of ^{111}In from prelabeled aortic endothelial cells (Table 2). Furthermore, TNF-α toxicity on actively growing endothelial cells was evaluated by counting cells in culture supernatants. Very few suspended cells were observed under such conditions regardless of TNF doses (up to 140 ng/ml) and incubation times (up to 5 days). TNF-α-induced inhibition of endothelial cell proliferation is reversible. Upon removal of TNF-α from cells incubated for 5 days with the inhibitor (1.4 ng/ml), cell growth in response to bFGF or serum was normal again (data not shown).

To test possible activity of TNF-α *in vivo*, we assessed its action on angiogenesis in the rabbit cornea. Since bFGF is a well-known angiogenesis factor in this animal model, the actions of TNF-α and bFGF were compared, and particularly it was tested if TNF-α inhibited FGF-induced neovascularization. Rabbit cornea angiogenesis induced by 0.5 μg of bFGF is shown in Fig. 4*d*. Unexpectedly, 0.5, 5, and 10 μg doses of TNF-α also caused an angiogenic response (see Fig. 4*c* for the 5 μg dose), with 0.5 μg representing a minimally active dose. The angiogenic response of 5 μg of TNF-α was comparable to that of 0.5 μg of bFGF. Despite the fact that TNF-α is an inhibitor of FGF-induced endothelial cell growth *in vitro*, it does not prevent the angiogenic response caused by bFGF. This was established by evaluating the effects of 0.1, 0.5, 5, and 10 μg of TNF-α in the presence of 0.5 μg of bFGF. Typical responses are shown in Fig. 4 *a* and *b*. The above described experiments were repeated with quantitatively identical results. TNF-α at concentrations of 5 μg or above evoked an inflammatory response, as evidenced by a cloudy cornea and a massive invasion of blood vessels from the limbus (Fig. 4*b*) and by histologic examination of epon-embedded corneas, which showed a large number of infiltrating leukocytes (data not shown). Furthermore, TNF-α-induced angiogenesis was associated with leaky blood vessels, as evidenced by minor hemorrhage surrounding the tips of newly formed capillaries. The inflammatory response to TNF-α occurred regardless of whether TNF-α was implanted alone or together with bFGF, which by itself does not cause inflammation.

Table 2. Effect of short-term TNF-α exposure on ^{111}In release by endothelial cells

TNF-α, ng/ml	% of maximal release, 4 hr	% of maximal release, 10 hr
0	8.5 ± 1.7	23.8 ± 4.0
1.4	3.7 ± 1.1	13.0 ± 0.6
140	4.0 ± 1.6	15.5 ± 4.0
Triton X-100	100 ± 0.6	ND

Values are means ± SD of triplicates and are expressed as percentage of maximal release (Triton X-100 treatment). ND, not determined.

DISCUSSION

The inhibitory or cytotoxic activity of TNF-α toward various tumor cell lines is well known (8, 9). The data presented here show that TNF-α is also a potent inhibitor of the *in vitro* growth of two types of vascular endothelial cells, confirming in part the results of other recent reports (18, 27). TNF-α inhibits with similar potency the growth of endothelial cells promoted by serum alone and the additional growth observed with growth factor-supplemented serum (aFGF and bFGF). This activity of TNF-α is not restricted to endothelial cells; arterial smooth muscle cell growth is also inhibited. However, the proliferation of several other normal cells is not inhibited by TNF-α (9, 10). Previous evidence obtained with tumor cell lines (8) but also with endothelial cells (18, 27, 38) suggests that the inhibitory activity of TNF-α may be largely due to cytotoxicity of this protein for those cell types. In our hands, two experiments designed at evaluating the cytotoxicity of TNF-α for endothelial cells show no indication of a toxic action: long-term incubation of cells with TNF-α does not cause overt cell loss nor does short-term exposure cause damage to the cell. Moreover, TNF-α action is reversible because treated cells resume normal growth upon removal of the inhibitor. The morphology of bovine endothelial cells was not altered by long-term exposure of confluent cells to high doses of TNF-α (data not shown), which is in contrast to previous findings with human and bovine endothelial cells (18, 27). The reasons for those discrepancies are unclear. It remains to be determined whether small experimental differences such as different culture conditions, differences in the origins of cells (human umbilical versus bovine aortic), or inhibitor (purified natural versus recombinant TNF-α) play a role.

Our data suggest that the inhibition of FGF-stimulated growth of endothelial cells is not mediated by a competition of TNF-α for the FGF receptor. Otherwise, very little is known with respect to the cellular mechanism of TNF-α inhibitory action on endothelial cells. Recently it was shown (17) that in fibroblasts TNF-α induces the expression of interferon-β_2 mRNA and protein, which presumably modulates cell growth. Since interferons have already been demonstrated to be inhibitory for endothelial cell growth (18, 39, 40), it will be of interest to establish whether a similar mechanism also works in those cells. Obviously, other mechanisms need to be considered as well, such as, for example, modulation of the expression of growth factor receptors and receptor down-regulation by TNF-α.

It should be noted that activities of TNF-α on endothelial cells described here resemble qualitatively those of another regulatory protein, transforming growth factor type β (TGF-β), which is also a highly potent, reversible, and noncytotoxic inhibitor of basal or stimulated endothelial cell growth (41–43). It is interesting that TNF-α and TGF-β are both bifunctional with respect to their activities on cell proliferation. Depending on cell types and culture conditions they can act either as growth stimulators or inhibitors (9, 44). TNF-α and TGF-β, as well as interferons, which are also inhibitory for endothelial cells (39), are well-established regulatory proteins, the physiological significance of which was originally thought to be associated with initially recognized biological activities—i.e., necrotic/cytotoxic, transforming, and antiviral activity, respectively. Recently it has become increasingly clear, however, that those factors are also antimitogenic for a variety of cell types (45). Although it is not possible to deduce physiological functions merely from *in vitro* experiments, the available evidence, nevertheless, lends some credibility to the hypothesis that TNF-α, TGF-β,

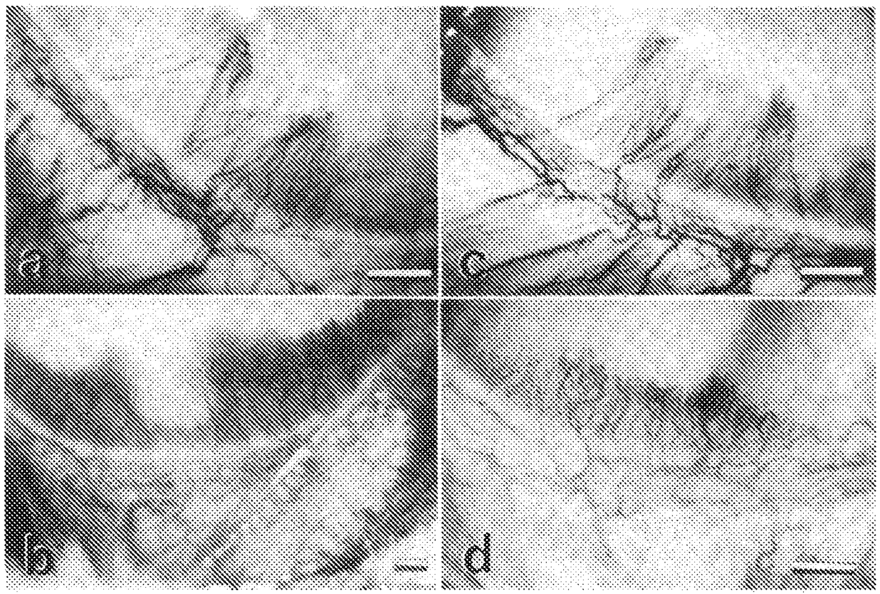


FIG. 4. Effect of TNF-α on angiogenesis in the rabbit cornea. Representative photographs of rabbit corneas implanted with Elvax pellets containing various doses of TNF-α and/or bFGF. (*a*) bFGF, 0.5 μg; TNF-α, 0.5 μg. (*b*) bFGF, 0.5 μg; TNF-α, 10 μg. (*c*) TNF-α, 5 μg. (*d*) bFGF, 0.5 μg. A total of 18 corneas were evaluated (3 with bFGF alone, 4 with TNF-α alone, 11 with combinations of bFGF and TNF-α). Negative controls were performed with rabbit serum albumin incorporated into Elvax pellets (37). Vascular sprouts were first seen 2 days after implantation of either FGF or TNF-α. Inflammatory angiogenesis was evident by a cloudy cornea and massive invasion of blood vessels as shown (*b*). Blood vessels induced by inflammation usually began to regress about 14 days after implantation. Photographs *a* and *c* and *b* and *d* were taken 16 and 11 days after implantation, respectively. (Bars = 1 mm.)

and interferons may possess as yet unrecognized physiological properties. It is conceivable, for example, that they fulfill a negative local regulatory role in the control of cell proliferation by counteracting the mitogenic activities of tissue growth factors—e.g., the omnipresent bFGF. TGF-β occurs rather ubiquitously in tissues. Interferon production can be induced in most cells and TNF-α is brought into tissues by means of activated macrophages. All three factors seem therefore strategically placed to act as local growth inhibitors.

We have explored this hypothesis by investigating whether TNF-α inhibits endothelial cell proliferation *in vivo* and, hence, neovascularization. An additional argument for those studies was the possibility that TNF-α-induced tumor necrosis may be, at least in part, a result of the inhibition of tumor neovascularization. In this context the observation of a stimulatory effect of TNF-α on angiogenesis in the rabbit cornea was surprising. The present data demonstrate that TNF-α causes the ingrowth of capillary blood vessels into the cornea and appears to enhance rather than inhibit bFGF-induced angiogenesis in the same *in vivo* model.

It is important to distinguish between TNF-α and the well-established angiogenesis factors such as bFGF (46). Although the latter induce capillary vessel formation in the absence of an inflammatory reaction, angiogenesis caused by TNF-α is accompanied by inflammation. Furthermore, TNF-α, especially at higher doses, causes newly formed blood vessels to leak, which is noticeable as a weak hemorrhage. It is well known that inflammation—i.e., the infiltration of macrophages into the inflammatory site—represents by itself an angiogenic stimulus. Presumably, macrophages can produce and release angiogenic factors such as bFGF (47) and possibly others of unknown nature. Alternative mechanisms, such as TNF-α-induced local production of angiogenic factors (e.g., prostaglandin E_2), should be investigated as well.

Finally, it is interesting to note that the resemblance between TNF-α and TGF-β *in vitro* extends to neovascularization *in vivo*. Like TNF-α, TGF-β stimulates angiogenesis (48). Moreover, with both proteins angiogenesis is associated with an inflammatory response. It is known that TGF-β is an extremely potent chemoattractant for macrophages (49). Thus, it is conceivable that TGF-β-induced neovascularization is a consequence of the release of angiogenic products from attracted macrophages. It remains to be seen whether a similar mechanism could be responsible for the angiogenic activity of TNF-α.

We thank T. Michel and Z.-P. Jiang for excellent technical assistance, H.-G. Zerwes for help with the cornea histology, and Drs. F. Frickel (Knoll GmbH, Ludwigshafen, Federal Republic of Germany) and D. Gospodarowicz (University of California, San Francisco) for generously supplying recombinant TNF and endothelial cells, respectively. We are indebted to Dr. P. Petrides (Munich, Federal Republic of Germany) for valuable suggestions. Research was supported by the Swiss National Science Foundation (Grant 3.649-0.84), the Kanton of Zürich, and the Hartmann-Müller and EMDO Foundations, Zürich.

1. Carswell, E. A., Old, L. J., Kassel, R. L., Green, S., Fiore, N. & Williamson, B. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 3666–3670.
2. Beutler, B., Greenwald, D., Hulmes, J. D., Chang, M., Pan, Y.-C. E., Mathison, J., Ulevitch, R. & Cerami, A. (1985) *Nature (London)* **316,** 552–554.
3. Pennica, D., Nedwin, G. E., Hayflick, J. S., Seeburg, P. H., Derynck, R., Palladin, T. A., Kohr, W. J., Aggarwal, B. B. & Goeddel, D. V. (1985) *Nature (London)* **312,** 724–729.
4. Gray, P. W., Aggarwal, B. B., Benten, C. V., Bringman, T. S., Henzel, W. J., Jarrett, J. A., Leung, D. W., Moffat, B., Ng, P., Sverdersky, L. P., Palladino, M. A. & Nedwin, G. E. (1984) *Nature (London)* **312,** 721–724.
5. Stone-Wolff, D. S., Yip, Y. K., Kelker, H. C., Le, J., Henriksen-DeStefano, D., Rubin, B. Y., Rinderknecht, E., Aggarwal, B. B. & Vilcek, J. (1984) *J. Exp. Med.* **159,** 828–843.
6. Beutler, B. & Cerami, A. (1986) *Nature (London)* **320,** 584–588.
7. Watanabe, N., Niitsu, Y., Sone, H., Neda, H., Yamauchi, N. & Urushizaki, I. (1985) *Jpn. J. Cancer Res.* **76,** 989–994.

8. Niitsu, Y., Watanabe, N., Sone, H., Neda, H., Yamauchi, N. & Urushizaki, I. (1985) *Jpn. J. Cancer Res.* **76**, 1193–1197.
9. Sugarman, B. J., Aggarwal, B. B., Hass, P. E., Figari, J. S., Palladino, M. A., Jr., & Shepard, H. M. (1985) *Science* **230**, 943–945.
10. Vilcek, J., Palombella, V. J., Henriksen-DeStefano, D., Swenson, C., Feinman, R., Hirai, M. & Tsujimoto, M. (1986) *J. Exp. Med.* **163**, 632–643.
11. Beutler, B., Mahoney, J., Le Trang, N., Pekala, P. & Cerami, A. J. (1985) *J. Exp. Med.* **161**, 984–995.
12. Bertolini, D. R., Nedwin, G. E., Bringman, T. S., Smith, D. D. & Mundy, G. R. (1986) *Nature (London)* **319**, 516–518.
13. Saklatvala, J. (1986) *Nature (London)* **322**, 547–549.
14. Bachwich, P. R., Chensue, S. W., Larrick, J. W. & Kunkel, S. L. (1986) *Biochem. Biophys. Res. Commun.* **136**, 94–101.
15. Dayer, J. M., Beutler, B. & Cerami, A. (1985) *J. Exp. Med.* **162**, 2163–2168.
16. Nawroth, P. P., Handley, D., Cassimeris, J., Chess, L. & Stern, D. M. (1986) *J. Exp. Med.* **163**, 1363–1375.
17. Kohase, M., Henriksen-DeStefano, D., May, L. T., Vilcek, J. & Sehgal, P. B. (1986) *Cell* **45**, 659–666.
18. Stolpen, A. H., Guinan, E. C., Fiers, W. & Pober, J. S. (1986) *Am. J. Pathol.* **123**, 16–24.
19. Munker, R., Gasson, J., Ogawa, M. & Koeffler, H. P. (1986) *Nature (London)* **323**, 79–82.
20. Wong, G. H. W. & Goeddel, D. (1986) *Nature (London)* **323**, 819–822.
21. Mestan, J., Digel, W., Mittnacht, S., Hillen, H., Blohm, D., Möller, A., Jacobsen, H. & Kirchner, H. (1986) *Nature (London)* **323**, 816–819.
22. Nawroth, P. P. & Stern, D. M. (1986) *J. Exp. Med.* **163**, 740–745.
23. Bevilacqua, M. P., Pober, J. S., Majeau, G. R., Fiers, W., Cotran, R. S. & Gimbrone, M. A., Jr. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 4533–4537.
24. Broudy, V. C., Kansanshy, K., Segal, G. M., Harlan, J. M. & Adamson, J. W. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 7467–7471.
25. Collins, T., Lapierre, L. A., Fiers, W., Strominger, J. L. & Pober, J. S. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 446–450.
26. Pober, J. S., Bevilacqua, M. P., Mendrick, D. L., Lapierre, L. A., Fiers, W. & Gimbrone, M. A. (1986) *J. Immunol.* **136**, 1680–1686.
27. Sato, N., Goto, T., Haranaka, K., Satomi, N., Nariuchi, N., Mano-Hirano, Y. & Sawasaki, Y. (1986) *J. Natl. Cancer Inst.* **76**, 1113–1121.
28. Gospodarowicz, D., Cheng, J., Lui, G. M., Baird, A. & Böhlen, P. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 6963–6967.
29. Böhlen, P., Esch, F., Baird, A. & Gospodarowicz, D. (1985) *EMBO J.* **4**, 1951–1956.
30. Böhlen, P., Baird, A., Esch, F., Ling, N. & Gospodarowicz, D. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 5364–5368.
31. Ross, R. (1971) *J. Cell Biol.* **50**, 172–186.
32. Voyta, J. C., Via, D. P., Butterfield, C. E. & Zetter, B. R. (1984) *J. Cell Biol.* **99**, 2034–2040.
33. Bertoni, G., Keist, R., Groscurth, P., Wyler, R., Nicolet, J. & Peterhans, E. (1985) *J. Immunol. Methods* **78**, 123–133.
34. Danpure, H. J., Osman, S. & Brady, F. (1982) *Br. J. Radiol.* **55**, 247–249.
35. Fehr, J., Moser, R., Leppert, D. & Groscurth, P. (1985) *J. Clin. Invest.* **76**, 535–542.
36. Gimbrone, M., Cotran, R. S., Leapman, S. B. & Folkman, J. (1974) *J. Natl. Cancer Inst.* **52**, 413–427.
37. Risau, W. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 3855–3859.
38. Kull, F. C. & Cuatrecasas, P. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 7932–7936.
39. Böhlen, P., Fràter-Schröder, M., Michel, T. & Jiang, Z.-P. (1987) in *Angiogenesis*, eds. Rifkin, D. & Klagsbrun, M. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 119–124.
40. Heyns, A. du P., Eldor, A., Vlodavsky, I., Kaiser, N., Fridman, R. & Panet, A. (1985) *Exp. Cell Res.* **161**, 297–306.
41. Fràter-Schröder, M., Müller, G., Birchmeier, W. & Böhlen, P. (1986) *Biochem. Biophys. Res. Commun.* **137**, 295–302.
42. Baird, A. & Durkin, T. (1986) *Biochem. Biophys. Res. Commun.* **138**, 476–482.
43. Heimark, R. L., Twardzik, D. R. & Schwartz, S. T. (1986) *Science* **233**, 1078–1080.
44. Holley, R. W., Baldwin, J. H., Greenfield, S. & Armour, R. (1985) in *Growth Factors in Biology and Medicine*, eds. Evered, D., Nugent, J. & Whelan, J. CIBA Foundation Symposium 116 (Pitman, London), pp. 241–252.
45. Hunter, T. (1986) *Nature (London)* **322**, 14–15.
46. Esch, F., Baird, A., Ling, N., Ueno, N., Hill, F., Denoroy, L., Klepper, R., Gospodarowicz, D., Böhlen, P. & Guillemin, R. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 6507–6511.
47. Baird, A., Mormède, P. & Böhlen, P. (1985) *Biochem. Biophys. Res. Commun.* **126**, 358–364.
48. Roberts, A. B., Sporn, M. B., Assoian, R. K., Smith, J. M., Roche, N. S., Wakefield, L. M., Heine, U. I., Liotta, L. A., Falanga, V., Kehrl, J. H. & Fauci, A. S. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 4167–4171.
49. Wahl, S. M., Hunt, D. A., Wakefield, L. M., McCartney-Francis, N., Wahl, L. M., Roberts, A. B. & Sporn, M. B. (1987) *Proc. Natl. Acad. Sci. USA*, in press.

W3

Am J Physiol Lung Cell Mol Physiol
280: L1273–L1281, 2001.

Gas 6 promotes Axl-mediated survival in pulmonary endothelial cells

AILEEN M. HEALY,[1] JOHN J. SCHWARTZ,[2] XIAHUI ZHU,[1] BRIAN E. HERRICK,[1]
BRIAN VARNUM,[3] AND HARRISON W. FARBER[1]
[1]*Pulmonary Center, Boston University School of Medicine, Boston 02118*
and [2]*Department of Biology, Massachusetts Institute of Technology,*
Cambridge, Massachusetts 02139; and [3]*Amgen Corporation, Thousand Oaks, California 91320*

Received 11 October 2000; accepted in final form 26 December 2000

Healy, Aileen M., John J. Schwartz, Xiahui Zhu, Brian E. Herrick, Brian Varnum, and Harrison W. Farber. Gas 6 promotes Axl-mediated survival in pulmonary endothelial cells. *Am J Physiol Lung Cell Mol Physiol* 280: L1273–L1281, 2001.—We examined Gas 6-Axl interactions in human pulmonary artery endothelial cells (HPAEC) and in Axl-transduced HPAEC to test Gas 6 function during endothelial cell survival. We identified the 5.0-kb Axl, 4.2-kb Rse, and 2.6-kb Gas 6 mRNAs in HPAEC. Immunoprecipitation and Western blotting confirmed the presence of these proteins. Gas 6 is present in cell-associated and secreted fractions of growth-arrested HPAEC, independent of cell density. In addition, the Axl receptor is constitutively phosphorylated in growth-arrested cultures, and exogenous Gas 6 enhanced Axl phosphorylation threefold. Gas 6 added to growth-arrested HPAEC resulted in a significant increase in cell number (1.5 nM Gas 6 increased cell number 35%). Flow cytometry revealed that Gas 6 treatment resulted in 28% fewer apoptosing cells. Transduction of a full-length Axl cDNA into HPAEC resulted in 54% fewer apoptosing cells after Gas 6 treatment. Collectively, the data demonstrate antiapoptotic activities for Gas 6 in HPAEC and suggest that Gas 6 signaling may be relevant to endothelial cell survival in the quiescent environment of the vessel wall.

Rse; apoptosis; signal transduction

THE QUIESCENT, NONTHROMBOGENIC phenotype of the vascular endothelium is essential to hemostasis. Under normal conditions, endothelial cell turnover in the vessel wall is relatively low compared with other somatic cell types. However, under certain pathological conditions (e.g., atherosclerosis, pulmonary hypertension, and thrombotic thrombocytopenic purpura), endothelial cell proliferation occurs (9, 18, 20). Endothelial proliferation is associated with increased apoptosis, which in turn generates a prothrombotic phenotype (5). Dysregulation of the endothelial cell phenotype implies that endogenous signaling pathways exist to control cell survival and thus maintain hemostasis.

Gas 6, the product of the growth arrest-specific gene 6, is a soluble factor implicated in the regulation of multiple cellular functions, including growth, survival, adhesion, and chemotaxis (2, 10, 12, 30, 31). Gas 6 signaling is transduced via ligation with three known receptor tyrosine kinases (RTK), Axl (also UFO and Ark) (33), Rse (also Sky, Brt, Tyro-3) (19), and Mer (29). In addition, Gas 6 function is cell-type specific. For example, Gas 6-Axl interactions result in mitogenic and antiapoptotic responses in NIH/3T3 fibroblasts and vascular smooth muscle cells (12, 13, 30, 31). However, Gas 6-Axl interactions mediate cellular aggregation in the murine myeloid 32D cells but show none of the mitogenic or survival activities found in other cell types (26).

There is increasing evidence to suggest that Gas 6 regulates important aspects of vessel wall function. In vascular smooth muscle cells grown in culture, Gas 6 is a growth-potentiating factor for the G protein-coupled receptor agonists thrombin and angiotensin II (30). Gas 6 also prevents growth arrest-induced death and promotes chemotaxis in vascular smooth muscle cells (10, 31). Gas 6 is expressed in vascular endothelial cells (24, 37) and inhibits granulocyte adhesion to activated endothelial cells in vitro (2). In human umbilical vein endothelial cells (HUVEC), Gas 6 promotes cellular viability in the absence of growth factors (34). In vivo, balloon catheterization of rat carotid arteries induces Gas 6 expression within the neointima (27), indicating that Gas 6 is positioned to regulate the vascular response to injury.

Recent studies have begun to address the function for each of the Gas 6 receptors. For example, mice containing targeted deletions of any one receptor, Axl, Rse, or Mer, reveal no overt phenotype (22). However, deletion of all three receptors results in viable animals with multiple abnormalities, the most prominent being male sterility, but noted among the various phenotypes was increased apoptosis in the vessel wall (22). Overexpression of the Axl receptor in cells of myeloid lineages results in a phenotype similar to non-insulin-dependent diabetes mellitus, likely the result of alterations in tumor necrosis factor-α production (1). In vivo, the Axl receptor has been identified in vascular

Address for reprint requests and other correspondence: A. M. Healy, Pulmonary Center R-3, Boston Univ. School of Medicine, 715 Albany St., Boston, MA 02118 (ahealy@bupula.bu.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

smooth muscle cells and capillary endothelial cells of synovial tissue obtained from patients with rheumatoid arthritis (34). Collectively, these results suggest that Gas 6-Axl interactions may be cell and tissue specific.

We asked whether Gas 6 regulates endothelial cell survival at growth arrest. To address this question, we characterized Axl, Rse, and Gas 6 expression in human pulmonary artery endothelial cells (HPAEC). We identified Axl and Rse expression in HPAEC and found that Axl is autophosphorylated in growth-arrested cells and exogenous Gas 6 enhances Axl phosphorylation 3.5-fold. We examined cell-associated and secreted Gas 6 expression and found that growth arrest induces Gas 6 secretion into the medium independent of cell density. Our data also demonstrate that exogenous Gas 6 promotes cellular viability but is not a growth-potentiating factor for the G protein-coupled agonist thrombin. Finally, Gas 6 inhibits programmed cell death in endothelial cells, and this response is enhanced in HPAEC that overexpress the Axl receptor. Thus the results of our studies suggest that Gas 6 functions as an antiapoptotic factor in pulmonary vascular endothelial cells through ligation with the high-affinity Axl receptor tyrosine kinase.

METHODS

Cells and culture conditions. HPAEC (Clonetics) were grown to confluence in growth medium (EBM, Clonetics) containing 1.38 μM hydrocortisone, 3 pM recombinant human epidermal growth factor (both from Clonetics), 10 μg/ml endothelial cell growth supplement (Sigma), 10,000 U/ml penicillin and streptomycin (Sigma) and supplemented with 10% fetal bovine serum (FBS, HyClone). At confluence, HPAEC were growth arrested by replacing serum-containing medium with serum-free medium supplemented with hydrocortisone, penicillin, and streptomycin and with or without recombinant human Gas 6 (Amgen), or human protein S (Enzyme Research Laboratories, South Bend, IN).

For proliferation assays, HPAEC were plated at 25×10^3 cells/cm^2 and incubated for 24 h in growth medium. Growth medium was removed, cells were washed 2× with PBS, and test medium containing 0.5% FBS supplemented with recombinant human Gas 6 (1–6 nM), human thrombin (0.1–10.0 U/ml, Enzyme Research Laboratories), or Gas 6 (1.5 nM) plus thrombin (0.1–10.0 U/ml) was added. Medium was exchanged daily, cells were trypsinized, and cell number was determined electronically (Coulter Counter, Hialeigh, FL).

PCR and Northern blot analysis. Total RNA was isolated from HPAEC and C57/Black mouse lung using the TRIzol reagent (GIBCO BRL) or the guanidinium thiocyanate method (7) and measured by optical density (260- to 280-nm absorbance ratio). RNA integrity was checked by electrophoresis through formaldehyde-agarose gels stained with 25 μM ethidium bromide. Poly(A)$^+$ RNA was purified from total RNA by oligo(dT) cellulose column chromatography (GIBCO BRL).

cDNA probes for Northern blot analysis were generated using RT-PCR with total RNA isolated from either U937 cells (for Axl sequences) or HPAEC. The primers were 5′-GCAGGCTGAAGAAAGTCCCTTCG and 3′-GCTGGCTGACCACTATCCAGTC for Axl; 5′-CTGCAGTGTGGAGGGGATGGAGG and 3′-GCCACACTGGCTGGGAGATCTCGG for Rse; 5′-CAATCTCTGTTGAGGAGCTGG and 3′-GACCACGTGCTCTTGGCCGTC for Gas 6, and 5′-CCTTCCTGGGCATGGAGTCCTG and 3′-GGAGCAATGATCTTGATCTTC for β-actin. PCR products were radiolabeled and hybridized to total RNA and poly(A)$^+$ RNA immobilized on nylon membranes (Amersham).

HPAEC metabolic labeling and Rse immunoprecipitation. Rse receptor biosynthesis was determined in HPAEC by metabolically labeling confluent cultures of cells with [^{35}S]methionine (ICN) for 4 h after a 1-h incubation in methionine-free medium (GIBCO BRL). HPAEC extracts were prepared to enrich for membrane and cytoplasmic proteins and exclude cell nuclei from the preparation. HPAEC extracts were prepared by standard techniques. Protein concentration was determined by the Bio-Rad protein assay, and equal concentrations of cellular extracts were used for immunoprecipitation. Rse was immunoprecipitated from extracts with a polyclonal antibody raised against the amino terminus of Rse and defined here as anti-Rse IgG (originally anti-Sky IgG, the generous gift of Dr. Kensako Mizuno, Kyushu University, Fukuoka, Japan) (35). Rabbit anti-Rse or normal rabbit serum immunoprecipitates were collected on protein A (A/G) agarose (Santa Cruz Biotechnology), and bound proteins, eluted with SDS buffer, were electrophoresed on 7.5% polyacrylamide gels and prepared for fluorography.

Immunoprecipitation and Western blot analysis of Axl and Gas 6. Cell lysates for cell-associated Axl and Gas 6 were prepared as described for biosynthetic labeling but without radioisotope. Gas 6 was identified in HPAEC-conditioned medium by collecting medium after 2, 4, and 5 days of serum depletion. Conditioned medium was concentrated 40-fold by centrifugation in a concentrator fitted with a YM-30 membrane (Amicon). Concentrated medium and cell lysates were prepared for electrophoresis through 10% polyacrylamide gels, transferred to nitrocellulose membranes (Schleicher and Schuell), and detected by successive incubations with anti-Gas 6 antibody (Amgen), anti-rabbit IgG labeled with horseradish peroxidase (Santa Cruz Biotechnology), and enhanced chemiluminescence (Pierce).

Western blot analysis for Axl detection was as described for Gas 6 except that the primary antibody was an affinity-purified, rabbit anti-Axl IgG (Amgen). Axl was also immunoprecipitated from cell lysates using a goat anti-Axl IgG (Santa Cruz Biotechnology). Alternatively, tyrosine-phosphorylated Axl receptor was immunoprecipitated from confluent HPAEC cultures serum deprived for 24 h and then left untreated or treated with Gas 6, protein S, or FBS for 5 min. Tyrosine-phosphorylated Axl was immunoprecipitated from cell lysates with the monoclonal anti-phosphotyrosine IgG clone 4G10 (Upstate Biotechnology). Blotted proteins were detected using either the rabbit anti-Axl IgG from Amgen or a second rabbit anti-Axl IgG (the generous gift of Dr. E. Liu, University of North Carolina, Chapel Hill, NC).

Apoptosis assays. DNA fragmentation was determined as described (16). Briefly, cells were grown to confluence in serum-containing medium, at which time serum was removed and cells were cultured for 72–120 h in serum-free medium with one medium exchange at 48 h. In all three apoptosis assays described below, each experiment included a negative and a positive control. Cells maintained in serum-containing medium were used as a negative control, and cells maintained in serum-containing medium supplemented with 1 μM staurosporine (Sigma), a protein kinase inhibitor that induces apoptosis, were used as a positive control (16). DNA was isolated from both floating cells, which were pelleted from test medium, and attached cells. The samples were electrophoresed through a 1.8% agarose gel containing 25 μM ethidium bromide.

For Hoechst staining, cells were grown to confluence on glass coverslips, serum deprived, and treated with various factors. Cells were fixed on *days 3* and *4* and stained simultaneously by inverting glass coverslips onto a drop of staining solution containing 4% formaldehyde, 0.6% Nonidet P-40, and 18.7 μM Hoechst 33258 (Sigma) in PBS at room temperature for 30 min (28). Fifty cells from three fields were counted for each condition in duplicate. Cells were scored as apoptotic if they displayed a highly condensed and fragmented nucleus.

Annexin V-positive- and propidium iodide-negative-stained HPAEC were detected by flow cytometry. HPAEC were grown as described previously except that cells were harvested on *days 2* and *3* of serum-free culture. Cells were trypsinized, counted, and costained with fluorescein-conjugated annexin V and propidium iodide (R&D Systems). Cells were analyzed by flow cytometry (Becton Dickinson) and quantitated using Cell Quest software.

Retroviral transduction of HPAEC with Axl constructs. A full-length cDNA encoding the Axl gene (gift of Dr. E. T. Liu) was subcloned into the *Eco*RI site of pMSCVpac (15). The Axl-retroviral construct was transfected into Phoenix cells to generate retroviral supernatants as previously described (6). Transduced HPAEC were selected by puromycin resistance and analyzed between *passages 6* and *9*.

Statistics. The data are expressed as means ± SD. Analysis of variance was carried out using the two-factor ANOVA. Statistical analysis comparing cells maintained in serum-free medium in the presence or absence of Gas 6 was conducted using Student's *t*-test. Differences were significant at $P < 0.05$.

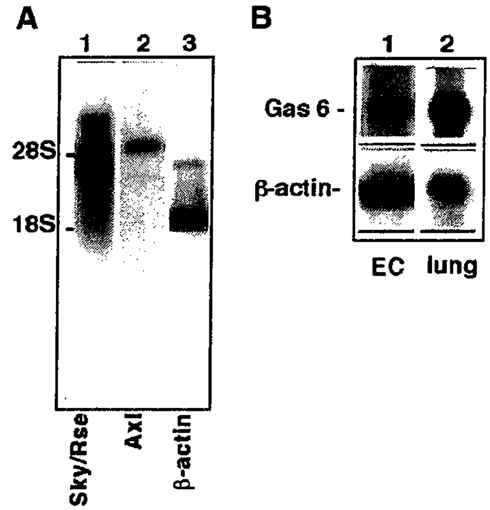


Fig. 1. Northern blot of receptor tyrosine kinases Rse and Axl and Gas 6 poly(A)$^+$ RNAs. *A*: human pulmonary artery endothelial cell (HPAEC) RNA was harvested and 2 μg of poly(A)$^+$ RNA were added to each lane, electrophoresed, and blotted to nitrocellulose. cDNA probes for Rse (*lane 1*), Axl (*lane 2*), and β-actin (*lane 3*) were successively hybridized to the same blot. The ribosomal bands are indicated as 28S and 18S. *B*: RNA was harvested, and 2 μg of poly(A)$^+$ RNA were added to each lane, electrophoresed, and blotted to nitrocellulose. cDNA probes for Gas 6 and β-actin were successively hybridized to the same blot. *Lane 1*, HPAEC RNA; *lane 2*, C57/Black mouse lung RNA. Northern blots shown are representative images from 3 independent experiments.

RESULTS

Vascular endothelial cells express the RTK Axl and Rse. We amplified a 193-bp Axl fragment, a 208-bp Rse fragment, and a 589-bp Gas 6 fragment from HPAEC by RT-PCR. Axl and Rse expression were confirmed by Northern blot analysis of HPAEC RNA (Fig. 1*A*). Northern blotting revealed the presence of a major band migrating at 4.2 kb for Rse mRNA. The Axl probe identified a single major transcript at 5 kb and a second transcript just visible at 3.4 kb. It is noteworthy that in transformed and tumorigenic cells, both the 5.0- and 3.4-kb Axl transcripts are represented equally (33). Quantitation of the 5.0-kb Axl and 4.2-kb Rse mRNAs compared with β-actin from the same cell sample demonstrates that Rse mRNA is 2.2 times more abundant than Axl mRNA in HPAEC.

Gas 6 is expressed in pulmonary endothelial cells in culture and in whole lung. Previous investigations identified the ligand Gas 6 in many cells and organs, but particularly high levels were identified in HUVEC and bovine aortic endothelial cells and in human and murine lungs (2, 24, 37). We examined Gas 6 expression in endothelial cells isolated from human pulmonary artery and tested whether our human cDNA Gas 6 probe also hybridizes to murine Gas 6. Northern blot analysis of Gas 6 transcripts (Fig. 1*B*) revealed the presence of a single major band migrating at ~2.6 kb in HPAEC and in whole lung extracts from C57/Black mice.

Immunoprecipitation and Western blot analysis of Axl and Rse. We examined cell lysates for the presence of the Axl and Rse receptors. Using three different Axl antibodies, we detected several forms of the immunoreactive Axl RTK. For example, with a rabbit polyclonal Axl antibody, the Axl receptor appears as a single major band with a relative mobility of 125 kDa and a second minor band with a relative mobility of 104 kDa (Fig. 2, *lane 1*). When Axl was immunoprecipitated using a goat polyclonal Axl antibody and then blotted with a second rabbit Axl antibody, the Axl receptor is seen as a doublet with a relative mobility of 140 and 110 kDa (Fig. 2, *lane 3*). A similar pattern was observed when cells were metabolically labeled and immunoprecipitated with these same two immunoreagents (not shown). Anti-Axl antibody premixed with a fivefold molar excess of an Axl-Fc fusion molecule failed to recognize all forms of the Axl receptor. Several forms of the Axl receptor, which correspond in relative mobility to those shown in Fig. 2, have been described in other cell types as the precursor (p104) Axl polypeptide and partial (p120) and fully glycosylated (p140) forms of Axl (32).

The Rse receptor was not detected by Western blot of HPAEC lysates; Rse was detected only by metabolically labeling cells before immunoprecipitation with an anti-Rse antibody. The Rse receptor is reported as a 140-kDa polypeptide in Rse-transfected Chinese hamster ovary cells, and this particular anti-Rse IgG also immunoprecipitates a Src kinase, a 60-kDa polypeptide (38). In HPAEC, immunoprecipitation with anti-

Rse IgG revealed the presence of three polypeptides with a relative mobility of 54, 131, and 181 kDa (Fig. 3, arrowheads).

Growth arrest induces Gas 6 secretion independent of cell density. Because Gas 6 expression is associated with growth arrest, we asked whether cell density affects Gas 6 expression and secretion. Therefore, we examined HPAEC cultures under sparse (8×10^3 cells/cm^2) and confluent (32×10^3 cells/cm^2) cell densities for cell-associated and soluble forms of Gas 6 (Fig. 4). We found that HPAEC maintained in culture under either serum-free or low-serum conditions (0.5% FBS) expressed Gas 6 regardless of cell density. Densitometric analysis from four independent experiments revealed that 1.1 ± 0.3 ng Gas 6 per 1×10^6 cells accumulates in the conditioned medium of confluent cultures. Densitometric analysis from two experiments revealed that 1.4 ± 0.1 ng Gas 6 per 1×10^6 cells accumulates in the conditioned medium of sparse cultures. The cell-associated forms of Gas 6 that were present at 2 and 4 days of serum deprivation correspond to the mature polypeptide, with a relative mobility of ~70 kDa, a higher molecular mass form at 110 kDa (probably a dimer), and a third immunoreactive species at 50 kDa (likely an intracellular precursor or degradation product) (Fig. 4). The 70-kDa form is the predominant form present in the conditioned medium of both sparse and confluent serum-deprived HPAEC at both 2 (data not shown) and 4 days of culture. The anti-Gas 6 antibody readily detects between 0.2 and 2 ng of the recombinant human Gas 6 (Fig. 4, *lanes 5–7*)

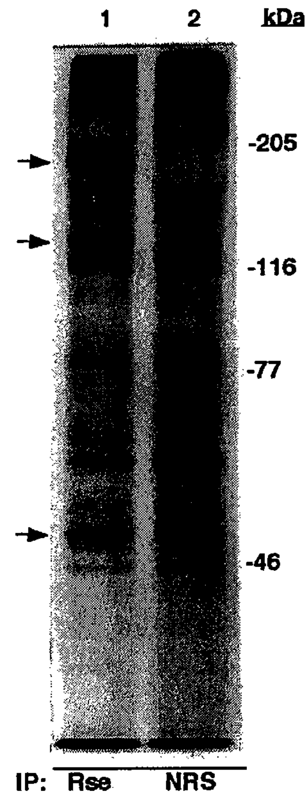


Fig. 3. Immunoprecipitation of the Rse receptor. HPAEC were grown to confluence and labeled with [^{35}S]methionine, and extracts were immunoprecipitated with anti-Rse antibody (Rse) or normal rabbit serum (NRS). The presence of 3 polypeptides specifically immunoprecipitated with anti-Rse antibody are indicated by arrowheads. Molecular mass markers (kDa) are indicated. A second independent experiment showed similar results.

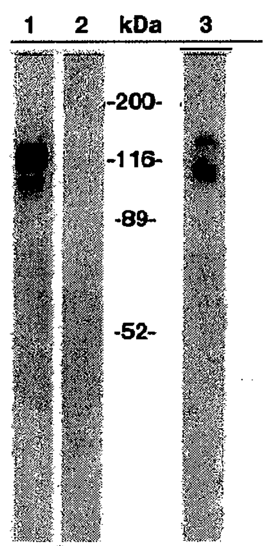


Fig. 2. Different forms of the Axl RTK are present in HPAEC. HPAEC were grown to confluence, and cell extracts were prepared, electrophoresed through a 7.5% polyacrylamide gel, and blotted. Four immunoreactive forms of the Axl RTK were detected by different anti-Axl antibodies. *Lane 1*, immunoblotting of HPAEC extracts (0.15×10^6 cells) with an anti-Axl IgG (Amgen); *lane 2*, competition with a soluble form of the Axl receptor; *lane 3*, immunoprecipitation and immunoblotting with 2 different anti-Axl antibodies (Santa Cruz Biotechnology and E. Liu). Molecular mass markers (kDa) are indicated. Images shown are representative of 3 independent experiments.

but does not cross-react with 20 ng of recombinant human protein S (data not shown).

Axl receptor is constitutively phosphorylated in HPAEC. The expression and secretion of Gas 6 in HPAEC led us to ask whether endogenous Gas 6 binds and activates its receptors. We found that the Axl receptor is phosphorylated in untreated cells (Fig. 5, *lane 1*). Moreover, the addition of exogenous Gas 6 (Fig. 5, *lane 2*) but not of serum (Fig. 5, *lane 3*) or protein S (data not shown) enhances Axl phosphorylation 3.5-fold. Phosphorylated Rse receptor was not detected (data not shown).

Gas 6 effects on HPAEC proliferation. In cell types that express Gas 6 plus both the Axl and Rse receptors, a mitogenic and/or antiapoptotic function for Gas 6 has been identified (10, 12, 21, 30, 31). Thus the presence of both the ligand Gas 6 and the two receptors Axl and Rse suggested that Gas 6 has proliferative and antiapoptotic properties in HPAEC. Our data show that the addition of recombinant human Gas 6 to HPAEC cultures results in a statistically significant increase in cell number (Fig. 6). The maximal increase in cell number occurred with exposure to 1.5 nM Gas 6 (100 ng/ml), resulting in a 36% increase in cell number. Higher concentrations of Gas 6, i.e., 3.0 and 6.0 nM (200 and 400 ng/ml) did not enhance the proliferative

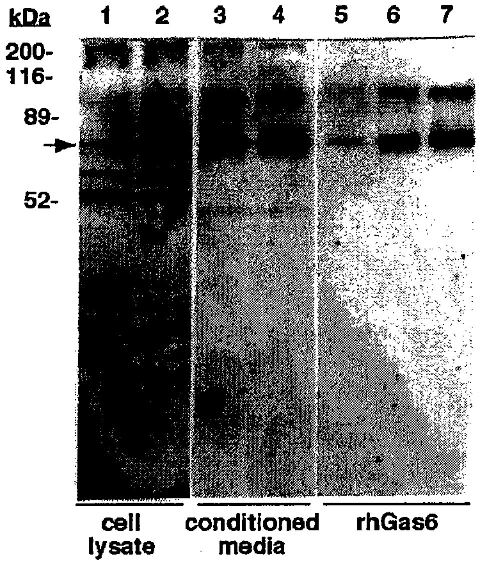


Fig. 4. Gas 6 is cell associated and released into conditioned medium. HPAEC were incubated for 4 days in serum-free medium. The immunoblot shows conditioned medium and cellular extracts after electrophoresis through a 10% polyacrylamide gel. *Lane 1*, Gas 6 detected by immunoblot of cell lysates from sparse cultures; *lane 2*, confluent cultures; *lane 3*, Gas 6 is also released into serum-free medium from sparse cultures; *lane 4*, confluent cultures; *lanes 5–7*, 0.2, 1, and 2 ng, respectively, of recombinant human Gas 6 (rhGasb). Gas 6 is indicated by arrow. Molecular mass markers (kDa) are indicated. Images shown are representative of 4 independent experiments (see text).

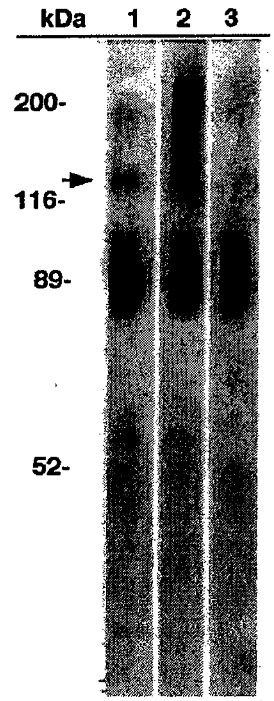


Fig. 5. Axl phosphorylation in growth-arrested HPAEC. HPAEC were grown to confluence in serum-containing growth medium. Quiescent HPAEC were either untreated or treated with Gas 6 or fetal bovine serum (FBS) for 5 min, lysed, and immunoprecipitated with the anti-phosphotyrosine antibody clone 4G10. Immunoprecipitated proteins were electrophoresed on a 7.5% polyacrylamide gel and immunoblotted with anti-Axl antibody. The Axl receptor is indicated by the arrowhead. Molecular mass markers (kDa) are shown. The results shown represent 3 independent experiments.

response further. In contrast, exposure to 10% FBS caused a 180% increase in cell number. The HPAEC response to Gas 6 stimulation is similar to previous findings by other investigators analyzing nonendothelial cell types (12, 21, 30).

It has been demonstrated previously that Gas 6 is a growth-potentiating factor for G protein-coupled receptor agonists such as thrombin and angiotensin II (21, 30). We tested thrombin alone (from 0.1 to 10.0 U/ml) and in combination with Gas 6 in our proliferation assays. However, neither thrombin alone nor in combination with 1.5 nM Gas 6 showed mitogenic or growth-potentiating activity (data not shown).

Apoptosis in HPAEC. Previous studies have identified antiapoptotic functions for Gas 6 in nonendothelial cells maintained under serum-free conditions (3, 12, 25, 31). HPAEC, like other endothelial cells, will apoptose if deprived of serum and growth factors. Therefore, we optimized the culture conditions to promote apoptosis in HPAEC before testing whether Gas 6 affects HPAEC survival.

In confluent cultures of HPAEC, DNA fragmentation is easily detected in cells maintained under serum-free culture conditions (Fig. 7, *lanes 4* and *5*) but not in cells grown in serum-containing medium (Fig. 7, *lane 3*). DNA fragmentation induced by staurosporine treatment is shown for comparison (Fig. 7, *lane 2*). These data confirm that serum-free culture conditions induce programmed cell death in HPAEC.

We used cellular morphology in conjunction with Hoechst staining to quantify attached apoptotic HPAEC and double labeling with annexin V and propidium iodide to quantify floating and attached apoptotic HPAEC. Gas 6 treatment results in a significant and reproducible survival effect on HPAEC maintained in serum-free conditions as measured by Hoechst staining and double labeling, as shown in Fig. 8. The addition of Gas 6 results in a 47% decrease in attached

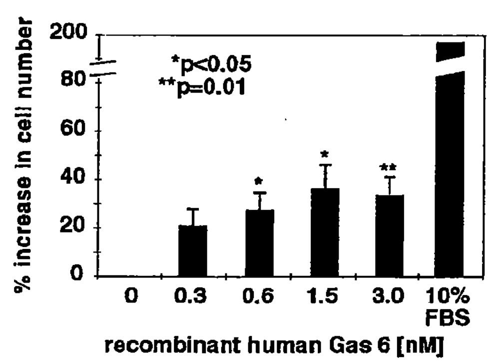


Fig. 6. Gas 6 increases HPAEC viability. HPAEC were plated at 25×10^3 cells/cm^2 and incubated for 24 h in growth medium. Growth medium was removed, and test medium containing 0.5% FBS with and without recombinant human Gas 6 (1–6 nM) was added. Cells maintained in 10% FBS were used as a control. Cell number was determined on *day 5*. The percent increase in cell number compared with untreated cells in shown. Values shown are means from 4 independent experiments.

Fig. 7. Serum depletion induces apoptosis in HPAEC. HPAEC were cultured for 4 days in the presence or absence of serum, and genomic DNA was isolated and electrophoresed on a 1% agarose gel containing ethidium bromide. *Lane 1*, DNA molecular mass marker; *lane 2*, DNA from HPAEC treated with staurosporine (positive control); *lane 3*, DNA from HPAEC grown in 10% serum (negative control), *lanes 4* and *5*, DNA from HPAEC maintained in serum-free medium. The results shown are representative of 4 independent experiments.

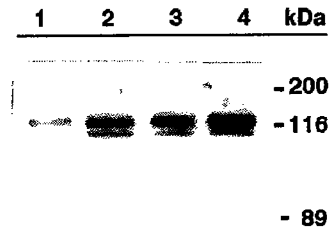


Fig. 9. Overexpression of Axl in transduced HPAEC. Nontransduced and Axl^{wt}-transduced HPAEC were grown to confluence, and cell extracts were prepared, electrophoresed through a 7.5% polyacrylamide gel, and blotted with an anti-Axl IgG (Amgen) as described. *Lanes 1* and *2*, nontransduced HPAEC extract (30 and 60 μg total protein per lane, respectively); *lanes 3* and *4*, Axl^{wt} HPAEC extract (30 and 60 μg total protein per lane, respectively). Molecular mass markers (kDa) are indicated. Images shown are representative of 3 independent experiments.

apoptosing HPAEC on *day 4* (Gas 6 10%, control 19%, $P < 0.001$) and a 28% decrease in floating plus attached HPAEC undergoing apoptosis on *day 2* (Gas 6 10%, control 14%, $P = 0.001$). Finally, under all the conditions shown in Fig. 8, there was no significant difference in the number of necrotic or dead cells (i.e., annexin V positive/propidium iodide positive) measured by flow cytometry (data not shown). Collectively, these results suggest that both the endogenous and exogenous Gas 6 function to inhibit HPAEC programmed cell death.

Axl mediates Gas 6 antiapoptotic function. The Axl receptor exhibits the highest affinity for Gas 6 compared with Rse and Mer (29). Therefore, to test Gas 6-receptor interactions during HPAEC survival, we generated Axl-transduced HPAEC using a full-length Axl cDNA (Axl^{wt}). We quantified Axl expression in transduced and nontransduced HPAEC by Western blot analysis and found a twofold increase in ectopic Axl expression (a representative blot is shown in Fig. 9). We used the Axl^{wt} HPAEC to test the effect of Gas 6 on cellular survival. We found that Gas 6 decreases the number of apoptotic Axl^{wt} HPAEC by 54% (Gas 6 5%, control 11%, $P < 0.05$) as shown in Fig. 10.

DISCUSSION

The vascular endothelium is a monolayer of contact-inhibited, growth-arrested cells lining the luminal sur-

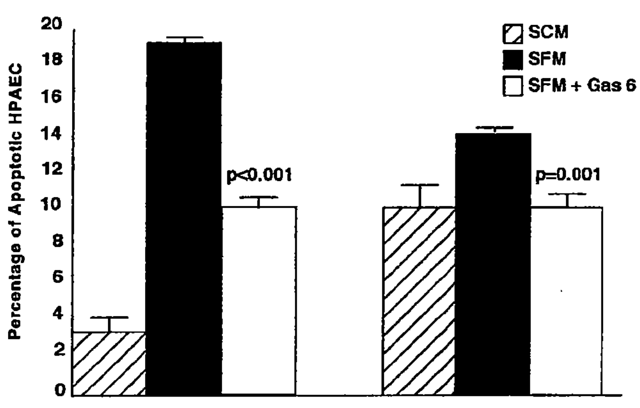


Fig. 8. Gas 6 is a survival factor for HPAEC. Confluent cultures of HPAEC were treated in either serum-containing medium (SCM, hatched bars), serum-free medium (SFM, solid bars), or serum-free medium supplemented with 1.5 nM recombinant human Gas 6 (open bars). Apoptosis was evaluated by Hoechst staining on *day 4* or by flow cytometry of annexin V-positive and propidium iodide-negative HPAEC on *day 2*. Values are the means from 3 independent experiments for Hoechst staining or 5 independent experiments for flow cytometry.

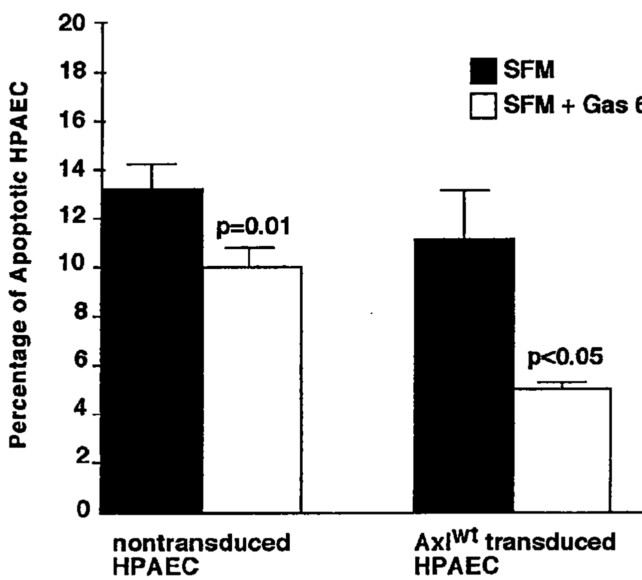


Fig. 10. Gas 6 promotes Axl-mediated survival. Confluent cultures of nontransduced HPAEC and Axl^{wt}-transduced HPAEC were treated for 2 days in serum-free medium (solid bars) or serum-free medium supplemented with 1.5 nM recombinant human Gas 6 (open bars). Apoptosis was evaluated by flow cytometry of annexin V-positive and propidium iodide-negative HPAEC. Values are the means from 4 independent experiments for flow cytometry.

face of the mature blood vessel wall. The molecular mechanisms contributing to the unique longevity of endothelial cells remain undefined. Gas 6 is a mitogen and survival factor for various cell types, transducing signals through its receptors Axl and Rse. To determine whether the Gas 6 signaling pathway is a potential mediator of endothelial cell survival at growth arrest, we examined the expression of Gas 6 and the receptors Axl and Rse and characterized the proliferative and antiapoptotic activities for Gas 6 in pulmonary endothelial cells in vitro.

We found that HPAEC simultaneously express both the Axl and Rse RTK. Axl and Rse receptors are detected from either total RNA or poly(A)$^+$ RNA, with a twofold higher steady-state level of Rse mRNA. Conversely, Western blot analysis indicates the Axl receptor is more highly expressed than the Rse receptor; this is most likely a reflection of differing antibody affinities rather than true differences in protein expression levels.

Gas 6 was originally identified as one of several molecules whose expression negatively correlates with cellular proliferation and serum depletion (24, 36). We questioned whether growth arrest by serum deprivation differs from growth arrest by contact inhibition in regard to Gas 6 expression and secretion. Measurement of Gas 6 levels in sparse vs. confluent cell cultures under serum-free conditions demonstrated no significant difference in cell-associated or soluble Gas 6 between the two cell densities, indicating that serum deprivation induces Gas 6 expression in vitro and contact-inhibited growth does not further augment Gas 6 expression. These results are in contrast to a recent study in which soluble Gas 6 was detected in the cell-associated fraction but not in the conditioned medium of HUVEC, suggesting that secreted Gas 6 may be completely bound to cell surface receptors (2). The difference between our findings and those of Avanzi et al. (2) may be due to the detection assays (i.e., Western blot vs. ELISA, respectively) or to the heterogeneity of endothelial cells isolated from different vascular beds. However, our data confirm a previous report demonstrating that Gas 6 is released into the conditioned medium from bovine aortic endothelial cells (37). Our results demonstrate that HPAEC growth arrested by either contact inhibition or serum depletion secrete Gas 6, which remains in a soluble form in the conditioned medium.

Previous studies revealed that Gas 6 is a growth-potentiating factor for the G protein-coupled receptor agonists such as thrombin and angiotensin II (21, 27, 30). Furthermore, it was shown that Gas 6 mitogenic activity is separable from Gas 6 antiapoptotic function; Gas 6 induces entry into the S phase of the cell cycle in the presence of low serum but is an antiapoptotic factor in the complete absence of serum (and growth factors) (3, 12). We found that at sparse cell densities in low-serum-containing medium, there is a statistically significant increase in HPAEC cell number in the presence of increasing concentrations of exogenous Gas 6. However, this proliferative response observed after 5 days of Gas 6 treatment may represent increased cell viability and not entry into S phase. The small increase in cell number (~5–7% per day) makes it difficult to test this hypothesis by standard techniques (e.g., measurement of [^{3}H]thymidine incorporation or 5-bromo-2′-deoxyuridine). In addition, we were unable to detect a Gas 6 growth-potentiating effect in the presence of thrombin. This finding supports the results of a previous study demonstrating that thrombin has a differential effect on endothelial cells isolated from distinct vascular beds and that long exposures to thrombin inhibit endothelial cell mitogenesis regardless of endothelial cell type (39). Our findings support the supposition that Gas 6 increases cell viability rather than stimulating mitosis in HPAEC.

Our data demonstrate that Gas 6 has an antiapoptotic function for HPAEC. Although the total population of HPAEC undergoing apoptosis on *day 2* (or *day 3*) of serum-free culture is relatively small (14% of total cells), the small number of apoptotic endothelial cells is in agreement with studies conducted on NIH/3T3 cells in which Gas 6 treatment decreased the number of apoptotic cells from ~11 to 4% (3). Hoechst staining revealed similar numbers of apoptosing HPAEC on *days 3* and *4* of serum-free culture. Furthermore, overexpression of the full-length Axl cDNA results in over a twofold increase in Axl protein levels and a corresponding decrease in the percentage of apoptotic cells. The results of studies examining apoptosis in the vessel wall in atherosclerotic lesions and regions of restenosis show a similar percentage of apoptotic cells, 2–30%, as detected by terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling (TUNEL) and Hoechst staining (11, 14, 17, 23). Moreover, in a single study addressing tumor angiogenesis, the complete removal of vascular endothelial growth factor resulted in the detachment of endothelial cells and subsequent tumor regression; however, only occasional TUNEL-positive endothelial cells could be identified within the blood vessel wall at any single time point (4). Thus the low numbers of HPAEC undergoing apoptosis in our studies are consistent with the results of studies performed on whole vessels. Collectively, our functional studies reveal that Gas 6 causes an increase in viability and a decrease in apoptosis, suggesting that Gas 6 is a survival factor for HPAEC.

Our studies indicate that the Axl receptor is constitutively phosphorylated and the addition of exogenous Gas 6, but not of serum or protein S, increases Axl phosphorylation 3.5-fold. These data indicate that Axl phosphorylation occurs via Gas 6 ligation. We also detected a 54-kDa protein that coprecipitates with the metabolically labeled Rse, which may be a member of the Src family of kinases (Fig. 3) (38), and a higher molecular mass band that may be a Rse-Src complex or a Rse-Gas 6 complex. These results support the supposition that Gas 6 promotes HPAEC survival through constitutive ligation with Axl and/or the Rse RTK.

It remains unknown whether Gas 6 interacts with both receptors or whether Axl and Rse can form heterodimers following ligand binding. The cell types

identified in which Gas 6 is a growth-potentiating and a survival factor express one or both receptors (Axl and Rse) in addition to the ligand (Gas 6) (12, 25, 31). These data support the hypothesis that the complex biology of the Gas 6 signaling pathway is regulated by cell type-specific expression of the Gas 6 receptors.

Our measurements indicate that picomolar concentrations of Gas 6 are synthesized by HPAEC under serum-free conditions. However, nanomolar concentrations are required for a cellular response in vitro, both in our studies and in independent studies of several cell types (3, 10, 12, 21, 25, 29). There are at least two possibilities that could explain this difference. Endogenous Gas 6-Axl interactions may not promote HPAEC survival. We think this is unlikely because gene deletion studies indicate that Axl-deficient embryonic fibroblasts are more susceptible to apoptosis after serum withdrawal and are refractory to exogenous Gas 6 treatment compared with Axl wild-type embryonic fibroblasts (3). Moreover, mice null mutant for all three Gas 6 receptors display increased TUNEL-positive cells in the vessel wall (22). We favor the supposition that the amount of endogenous Gas 6 may be limiting under our defined experimental conditions, and, therefore, endogenous Gas 6 cannot completely protect from apoptosis after serum withdrawal. This scenario would explain why we do not observe an increased cell survival in the Axl^{wt} HPAEC on serum withdrawal but do observe a twofold increase in survival after addition of exogenous Gas 6.

Programmed cell death is an integral component of the vascular response to injury. On the one hand, apoptosis in vascular smooth muscle cells counters the exuberant cellular proliferation that leads to intimal thickening (8, 18). On the other hand, apoptosis in vascular endothelium contributes to pathogenesis by promoting intravascular coagulation activation (5). Apoptosis also has a role in the vascular remodeling associated with tumor angiogenesis (4). Thus a balance between cell growth and cell death may be required for vascular remodeling. In this report, we characterized the expression and function of the Gas 6 signaling pathway in pulmonary endothelium in vitro. Further elucidation of this pathway will reveal whether Gas 6 functions in maintaining the equilibrium between cell growth and survival in lung endothelium in vivo.

We thank Dr. S. M. Notarnicola for a critical reading of the manuscript, Dr. R. D. Rosenberg for the transduction technology, and Laura Morgenthau for expert technical assistance.

This work was supported by National Heart, Lung, and Blood Institute Grant HL-45537 to H. W. Farber.

REFERENCES

1. **Augustine KA, Rossi R, Van G, Housman J, Stark K, Danilenko D, Varnum B, and Medlock E.** Noninsulin-dependent diabetes mellitus occurs in mice ectopically expressing the human Axl tyrosine kinase receptor. *J Cell Physiol* 181: 433–447, 1999.
2. **Avanzi GC, Callicchio M, Bottarel F, Gammaitoni L, Cavalloni G, Garbarino G, Varnum B, Aglietta M, Scagli G, Dianzani U, and Cianzani C.** Gas 6 inhibits granulocyte adhesion to endothelial cells. *Blood* 91: 2334–2340, 1998.
3. **Bellosta P, Zhang Q, Goff SP, and Basilico C.** Signaling through the ARK tyrosine kinase receptor protects from apoptosis in the absence of growth stimulation. *Oncogene* 15: 2387–2397, 1997.
4. **Benjamin LE and Keshet E.** Conditional switching of vascular endothelial growth factor (VEGF) expression in tumors. *Proc Natl Acad Sci USA* 94: 8761–8766, 1997.
5. **Bombeli T, Karsan A, Tait JF, and Harlan JM.** Apoptotic vascular endothelial cells become procoagulant. *Blood* 89: 2429–2442, 1997.
6. **Chatterton JE, Hirsch D, Schwartz JJ, Bickel PE, Rosenberg RD, Lodish HF, and Krieger M.** Expression cloning of LSLB, a gene essential for normal Golgi function and assembly of the ldlCp complex. *Proc Natl Acad Sci USA* 96: 915–920, 1999.
7. **Chomczynski P and Sacchi N.** Single step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 162: 156–159, 1987.
8. **Cowan KN, Heilbut A, Humpl T, Lam C, Ito S, and Rabinovitch M.** Complete reversal of fatal pulmonary hypertension in rats by a serine elastase inhibitor. *Nat Med* 6: 698–702, 2000.
9. **Dang CT, Magid MS, Weksler B, Chadburn A, and Laurence J.** Enhanced endothelial cell apoptosis in splenic tissues of patients with thrombotic thrombocytopenic purpura. *Blood* 93: 1264–1270, 1999.
10. **Fridell Y-WC, Villa J, Attar EC, and Liu ET.** Gas 6 induces Axl-mediated chemotaxis of vascular smooth muscle cells. *J Biol Chem* 273: 7123–7126, 1998.
11. **Geng Y-J and Libby P.** Evidence for apoptosis in advanced human atheroma. *Am J Pathol* 147: 251–266, 1995.
12. **Goruppi S, Ruaro E, and Schneider C.** Gas 6, the ligand of Axl tyrosine kinase receptor, has mitogenic and survival activities for serum starved NIH3T3 fibroblasts. *Oncogene* 12: 471–480, 1996.
13. **Goruppi S, Ruauo E, Varnum B, and Schneider C.** Requirement of phosphatidylinositol 3-kinase-dependent pathway and Src for Gas6-Axl mitogenic and survival activities in NIH 3T3 fibroblasts. *Mol Cell Biol* 17: 4442–4453, 1997.
14. **Han DKM, Haudenschild CC, Hong MK, Tinkle BT, Leon MB, and Liau G.** Evidence for apoptosis in human atherogenesis and in a rat vascular injury model. *Am J Pathol* 147: 267–277, 1995.
15. **Hawley RG, Lieu FH, Fong AZ, and Hawley TS.** Versatile retroviral vectors for potential use in gene therapy. *Gene Ther* 1: 136–138, 1994.
16. **Hogquist KA, Nett MA, Unanue ER, and Chaplin DD.** Interleukin 1 is processed and released during apoptosis. *Proc Natl Acad Sci USA* 88: 8485–8489, 1991.
17. **Isner JM, Kearney M, Bortman S, and Passeri J.** Apoptosis in human atherosclerosis and restenosis. *Circulation* 91: 2703–2711, 1995.
18. **Kockx MM and German AG.** Apoptosis in atherosclerosis: beneficial or detrimental? *Cardiovasc Res* 45: 736–746, 2000.
19. **Lai C and Lemke G.** An extended family of protein-tyrosine kinase genes differentially expressed in the vertebrate nervous system. *Neuron* 6: 691–704, 1991.
20. **Lee SD, Shroyer KR, Markham NE, Cool CD, Voelkel NF, and Tuder RM.** Monoclonal endothelial cell proliferation is present in primary but not secondary pulmonary hypertension. *J Clin Invest* 101: 927–934, 1998.
21. **Li R, Chen J, Hammonds G, Phillips H, Armanini M, Wood P, Bunge R, Godowski PJ, Sliwkowski MX, and Mather JP.** Identification of Gas6 as a growth factor for human Schwann cells. *J Neurosci* 16: 2012–2019, 1996.
22. **Lu Q, Gore M, Zhang Q, Camenisch T, Boast S, Casagranda F, Lai C, Skinner M, Klein R, Matsushima GK, Earp HS, Goff SP, and Lemke G.** Tyro-3 family receptors are essential regulators of mammalian spermatogenesis. *Nature* 398: 723–728, 1999.
23. **Malik N, Francis SE, Holt CM, Gunn J, Thomas GL, Shepard L, Chamberlain J, Newman CM, Cumberland DC, and Crossman DC.** Apoptosis and cell proliferation after porcine coronary angioplasty. *Circulation* 20: 1657–1665, 1998.
24. **Manfioletti G, Brancolini C, Avanzi G, and Schneider C.** The protein encoded by a growth arrest-specific gene (gas 6) is a

new member of the vitamin K-dependent proteins related to protein S, a negative coregulator in the blood coagulation cascade. *Mol Cell Biol* 13: 4976–4985, 1993.

25. **Matsubara N, Takahashi Y, Nishina Y, Mukouyama M, Watanabe T, Nakano T, Nomura K, Arita H, Nishimune Y, Obinata M, and Matsui Y.** A receptor tyrosine kinase, sky, and its ligand, Gas 6, are expressed in gonads and support primordial germ cell growth or survival in culture. *Dev Biol* 180: 499–510, 1996.
26. **McCloskey P, Fridell YW, Attar E, Villa J, Jin Y, Varnum B, and Liu ET.** Gas 6 mediates adhesion of cells expressing the receptor tyrosine kinase Axl. *J Biol Chem* 272: 23285–23291, 1997.
27. **Melaragno MG, Wuthrich DA, Poppa V, Gill D, Lindner V, Berk BC, and Corson MA.** Increased expression of Axl tyrosine kinase after vascular injury and regulation by G protein-coupled receptor agonists in rats. *Circ Res* 83: 697–704, 1998.
28. **Morin PJ, Vogelstein B, and Knizler KW.** Apoptosis and APC colorectal tumorigenesis. *Proc Natl Acad Sci USA* 93: 7950–7954, 1996.
29. **Nagata K, Ohashi K, Nakano T, Arita H, Zong C, Hanafusa H, and Mizuno K.** Identification of the product of growth arrest-specific gene 6 as a common ligand for Axl, Sky and Mer receptor tyrosine kinases. *J Biol Chem* 271: 30022–30027, 1996.
30. **Nakano T, Higashino K, Kikuchi N, Kishino J, Nomura K, Fujita H, and Arita H.** Vascular smooth muscle cell-derived, Gla-containing growth-potentiating factor for Ca2+-mobilizing growth factors. *J Biol Chem* 270: 5702–5705, 1995.
31. **Nakano T, Kawamoto K, Higashino K-I, and Arita H.** Prevention of growth-arrest induced cell death of vascular smooth muscle cells by a product of growth arrest-specific gene, gas6. *FEBS Lett* 387: 78–80, 1996.
32. **O'Bryan JP, Fridell Y-W, Koski R, Varnum B, and Liu ET.** The transforming receptor tyrosine kinase, Axl, is posttranslationally regulated by proteolytic cleavage. *J Biol Chem* 270: 551–557, 1995.
33. **O'Bryan JP, Frye RA, Cogswell PC, Neubauer A, Kitch B, Prokop C, Espinosa R, Le Beau MM, Earp HS, and Liu ET.** Axl, a transforming gene isolated from primary human myeloid leukemia cells, encodes a novel receptor tyrosine kinase. *Mol Cell Biol* 11: 5016–5031, 1991.
34. **O'Donnell K, Harkes IC, Dougherty L, and Wicks IP.** Expression of receptor tyrosine kinase Axl and its ligand Gas 6 in rheumatoid arthritis. *Am J Pathol* 154: 1171–1180, 1999.
35. **Ohashi K, Nagata K, Toshima J, Nakano T, Arita H, Tsuda H, Suzuki K, and Mizuno K.** Stimulation of Sky receptor tyrosine kinase by the product of the growth arrest-specific gene 6. *J Biol Chem* 270: 22681–22684, 1995.
36. **Schneider C, King RM, and Philipson L.** Genes specifically expressed at growth arrest of mammalian cells. *Cell* 54: 787–793, 1988.
37. **Stitt TN, Conn G, Gore M, Lai C, Bruno J, Radziejewski C, Jones PF, Masiakowski P, Ryan T, Lemke G, Glass DJ, and Yancopoulos GD.** The anticoagulation factor protein S and its relative, gas 6, are ligands for the tyro3/Axl family of receptor tyrosine kinases. *Cell* 80: 661–670, 1995.
38. **Toshima J, Ohashi K, Iwashita S, and Mizuno K.** Autophosphorylation activity and association with Src family kinase of Sky receptor tyrosine kinase. *Biochem Biophys Res Commun* 209: 656–663, 1995.
39. **Wang H-S, Li F, Runge M, and Chaikof EL.** Endothelial cells exhibit differential chemokinetic and mitogenic responsiveness to α-thrombin. *J Surg Res* 68: 139–144, 1997.

US 20040077574A1

(19) **United States**

(12) **Patent Application Publication**

Klinghoffer et al.

(10) **Pub. No.: US 2004/0077574 A1**

(43) **Pub. Date: Apr. 22, 2004**

(54) **MODULATION OF BIOLOGICAL SIGNAL TRANSDUCTION BY RNA INTERFERENCE**

(75) Inventors: **Richard Klinghoffer**, Seattle, WA (US); **Stephen Patrick Lewis**, Mountlake Terrace, WA (US)

Correspondence Address:
SEED INTELLECTUAL PROPERTY LAW GROUP PLLC
701 FIFTH AVE
SUITE 6300
SEATTLE, WA 98104-7092 (US)

(73) Assignee: **CEPTYR, Inc.**, Bothell, WA (US)

(21) Appl. No.: **10/444,795**

(22) Filed: **May 23, 2003**

Related U.S. Application Data

(60) Provisional application No. 60/462,942, filed on Apr. 14, 2003. Provisional application No. 60/383,249, filed on May 23, 2002.

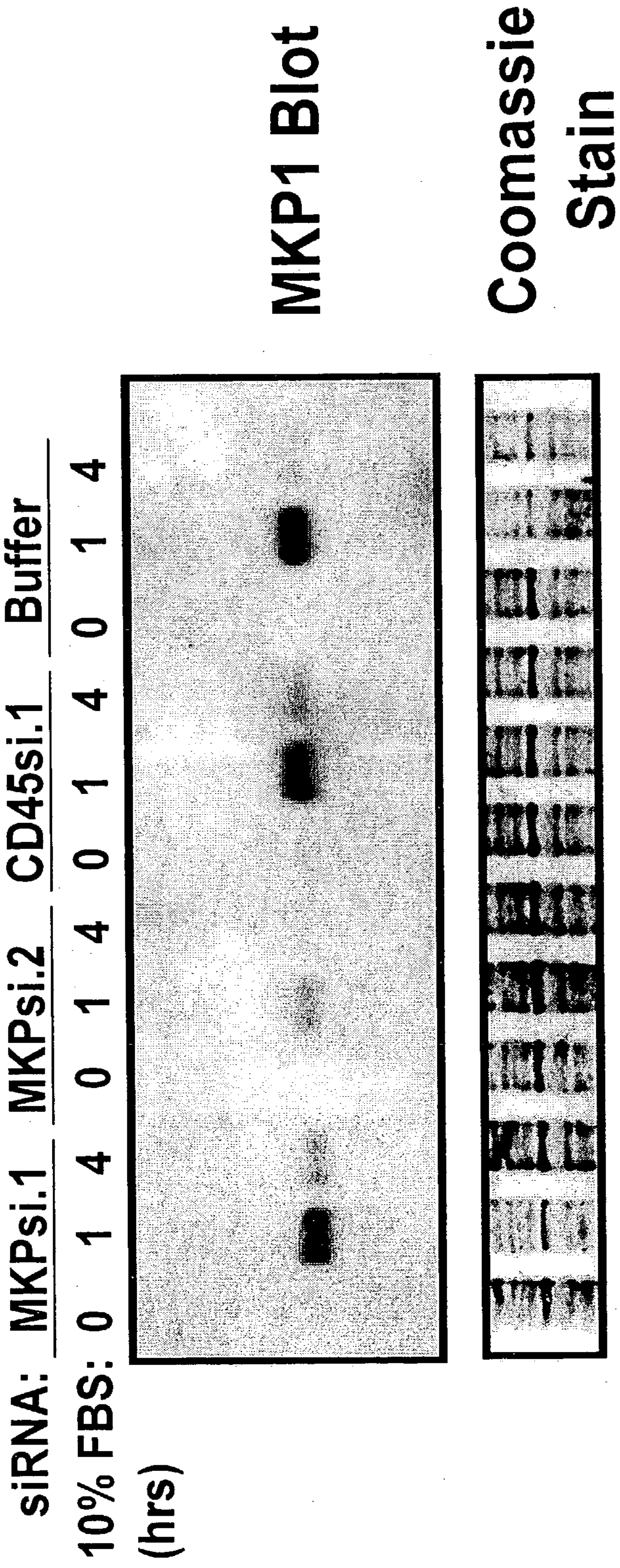
Publication Classification

(51) Int. Cl.^{7} **A61K 48/00**; C07H 21/02; C12N 15/85

(52) U.S. Cl. **514/44**; 435/455; 536/23.1

(57) **ABSTRACT**

Compositions and methods relating to small interfering RNA (siRNA) polynucleotides are provided as pertains to modulation of biological signal transduction. Shown are siRNA polynucleotides that interfere with expression of members of the protein tyrosine phosphatase (PTP) class of enzymes that mediate signal transduction, and with certain MAP kinase kinases (MKK). In certain preferred embodiments siRNA modulate signal transduction pathways comprising SHP2, cdc14a/b, cdc25A/B/C, KAP, PTP-ε, PRL-3, CD45, dual specificity phosphatase-3 (DSP-3), MKK-4, and/or MKK-7. Modulation of PTP-mediated biological signal transduction has uses in diseases associated with defects in cell proliferation, cell differentiation and/or cell survival, such as metabolic disorders (including diabetes and obesity), cancer, autoimmune disease, infectious and inflammatory disorders and other conditions. The invention also provides siRNA polynucleotides that interfere with expression of chemotherapeutic target polypeptides, such as DHFR, thymidylate synthetase, and topoisomerase I.



HeLa cells, transfected with siRNA duplexes 24 hr before stimulation with FBS.

Fig. 1

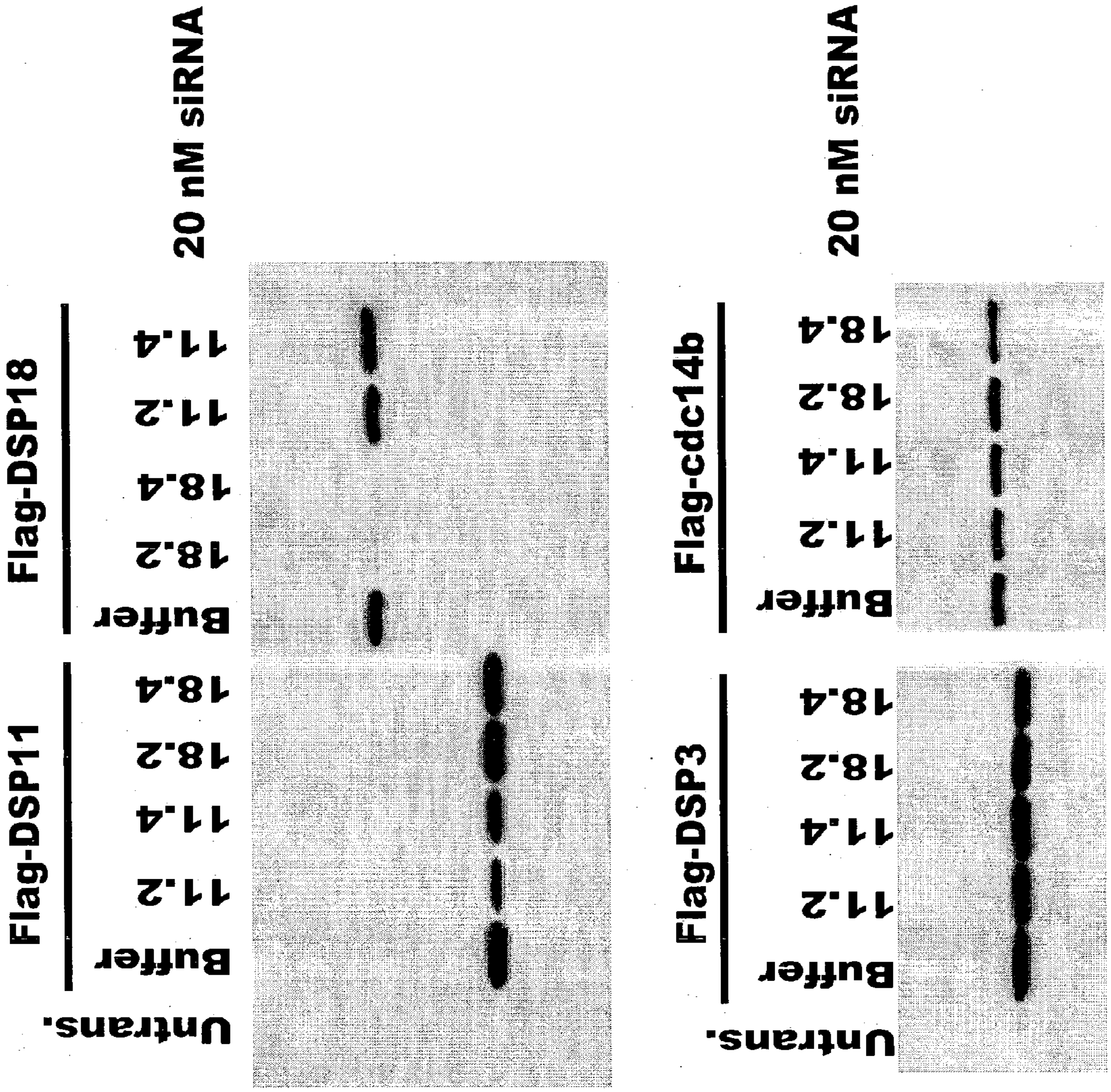

20 nM siRNA
Flag-DSP18
11.4
11.2
18.4
18.2
Buffer
Flag-DSP11
18.4
18.2
11.4
11.2
Buffer
Untrans.
20 nM siRNA
Flag-cdc14b
18.4
18.2
11.4
11.2
Buffer
Flag-DSP3
18.4
18.2
11.4
11.2
Buffer
Untrans.

Fig. 2

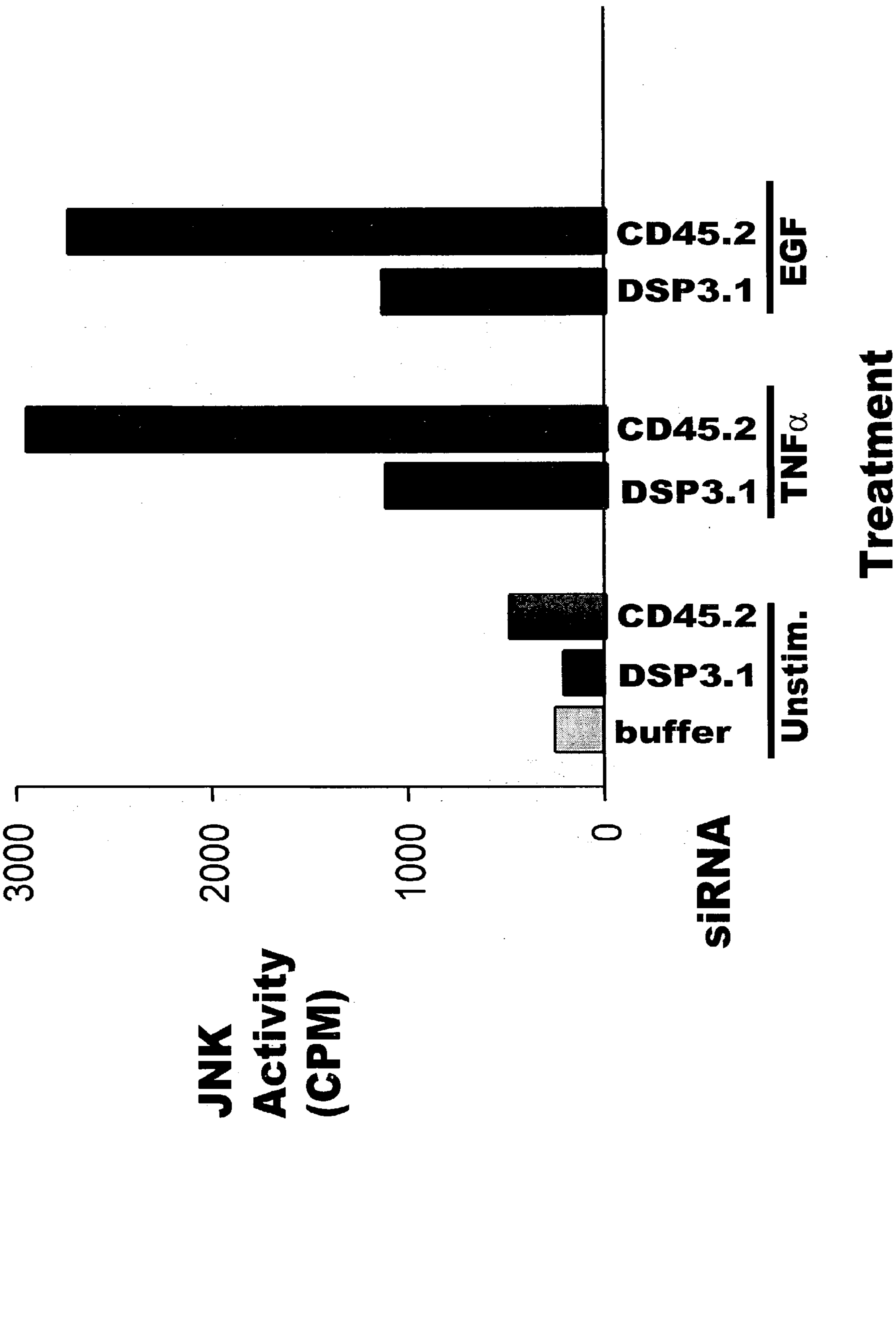

JNK Activity (CPM)
3000
2000
1000
0
siRNA
buffer
DSP3.1
CD45.2
Unstim.
DSP3.1
CD45.2
TNFα
DSP3.1
CD45.2
EGF
Treatment

Fig. 3

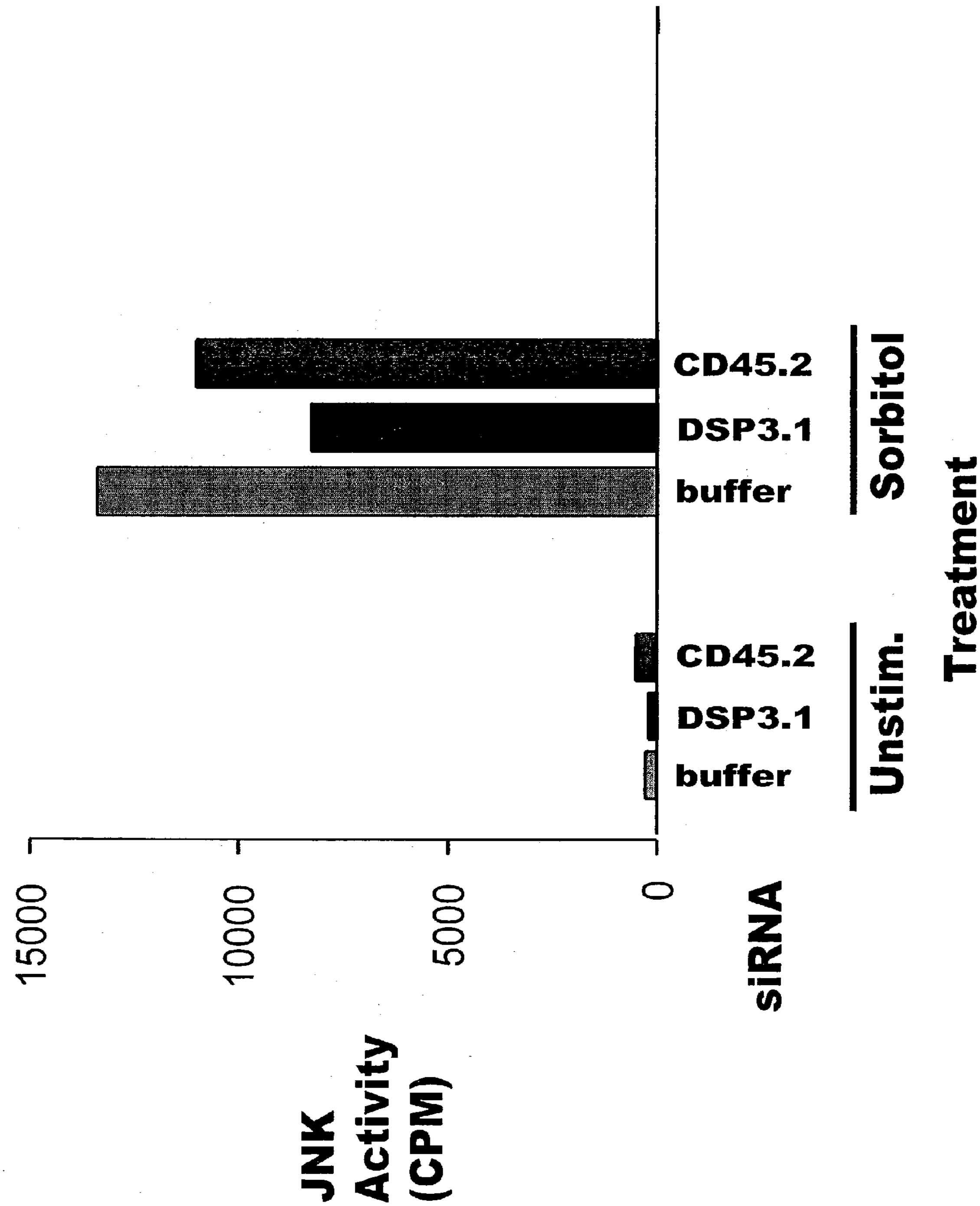

JNK Activity (CPM)
15000
10000
5000
0
siRNA
buffer
DSP3.1
CD45.2
Unstim.
buffer
DSP3.1
CD45.2
Sorbitol
Treatment

Fig. 4

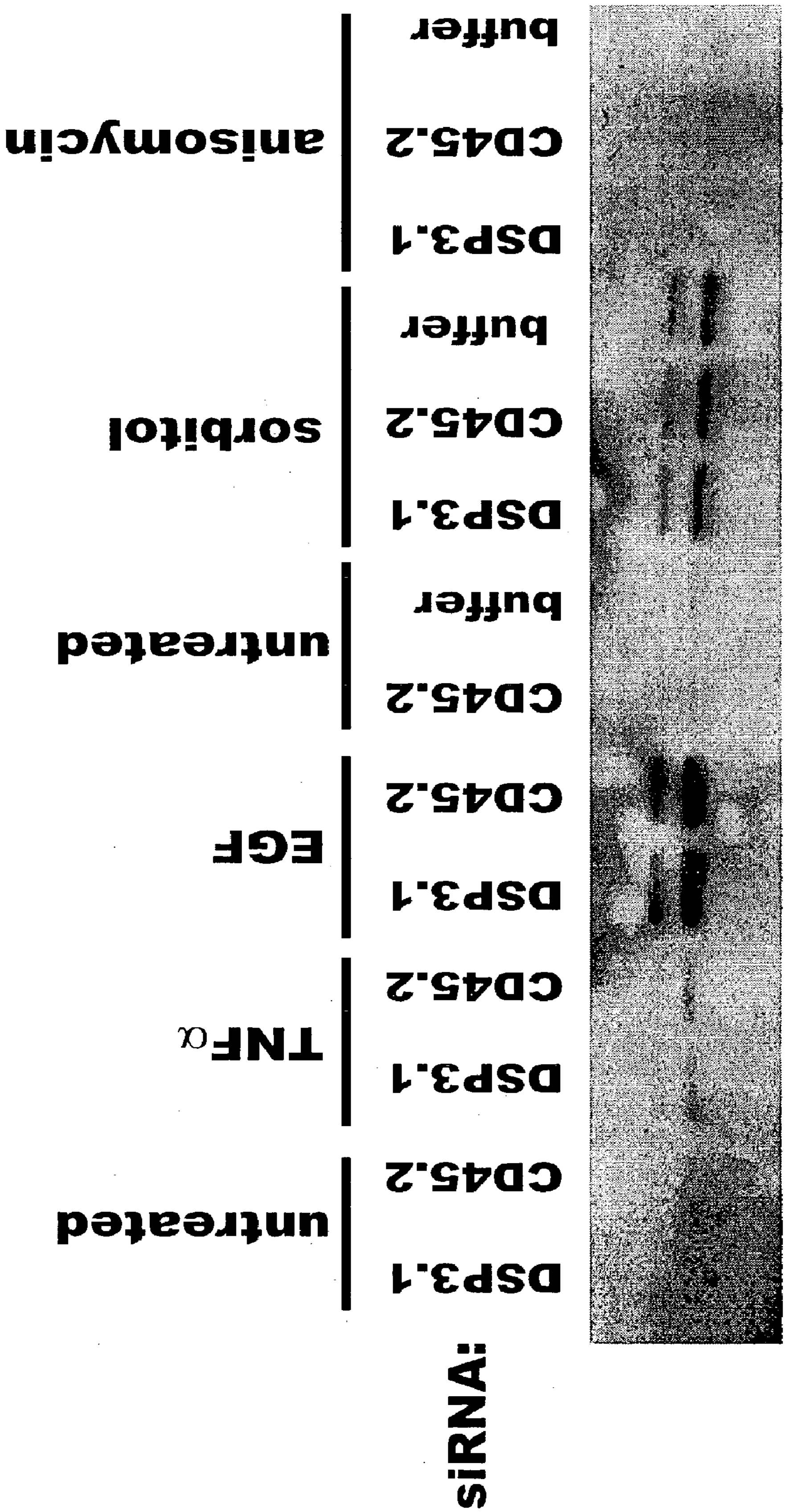

siRNA:
untreated
DSP3.1
CD45.2
TNFα
DSP3.1
CD45.2
EGF
DSP3.1
CD45.2
untreated
CD45.2
buffer
sorbitol
DSP3.1
CD45.2
buffer
anisomycin
DSP3.1
CD45.2
buffer

Fig. 5

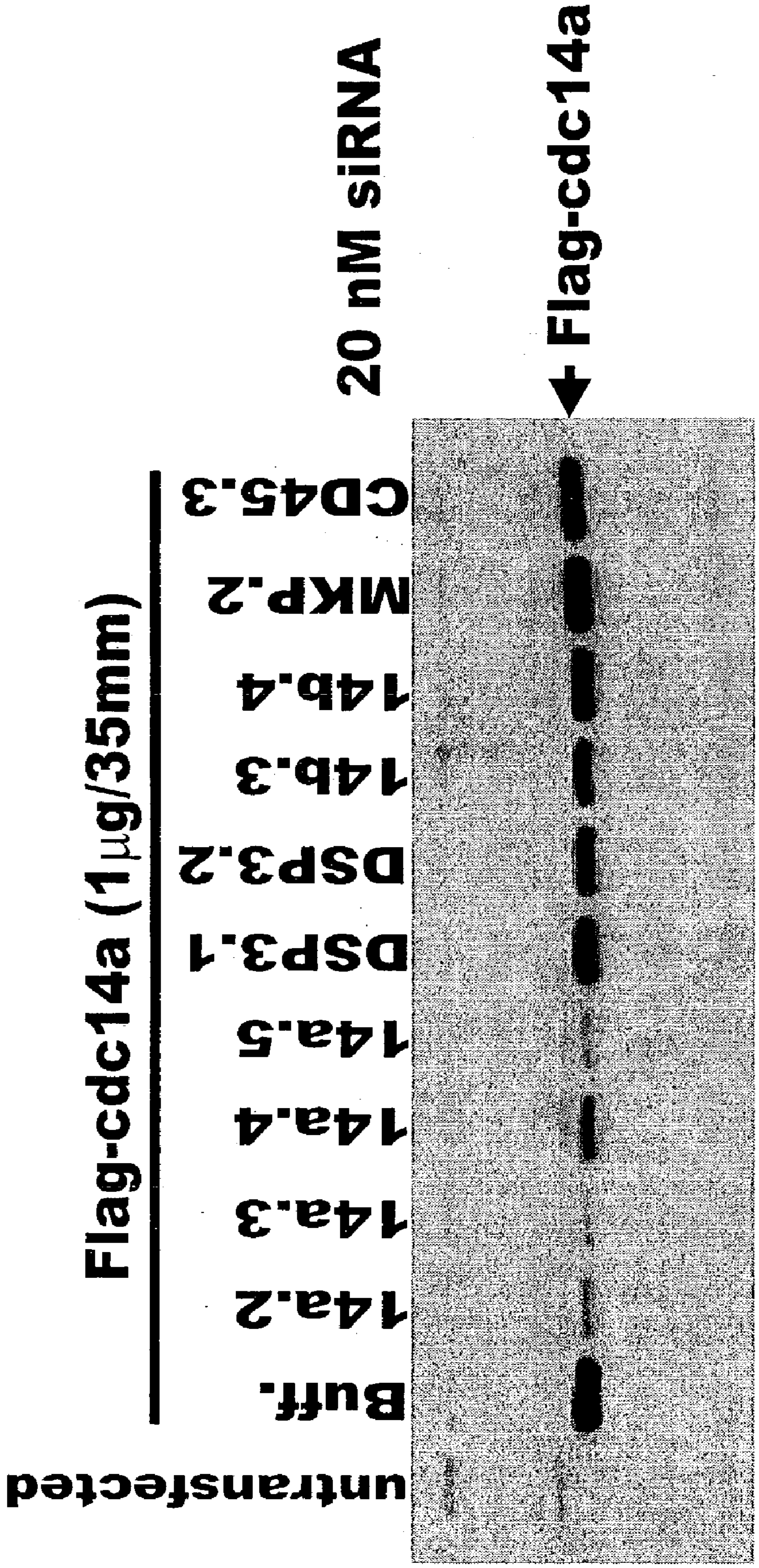

20 nM siRNA
Flag-cdc14a
Flag-cdc14a (1μg/35mm)
CD45.3
MKP.2
14b.4
14b.3
DSP3.2
DSP3.1
14a.5
14a.4
14a.3
14a.2
Buff.
untransfected

Fig. 6

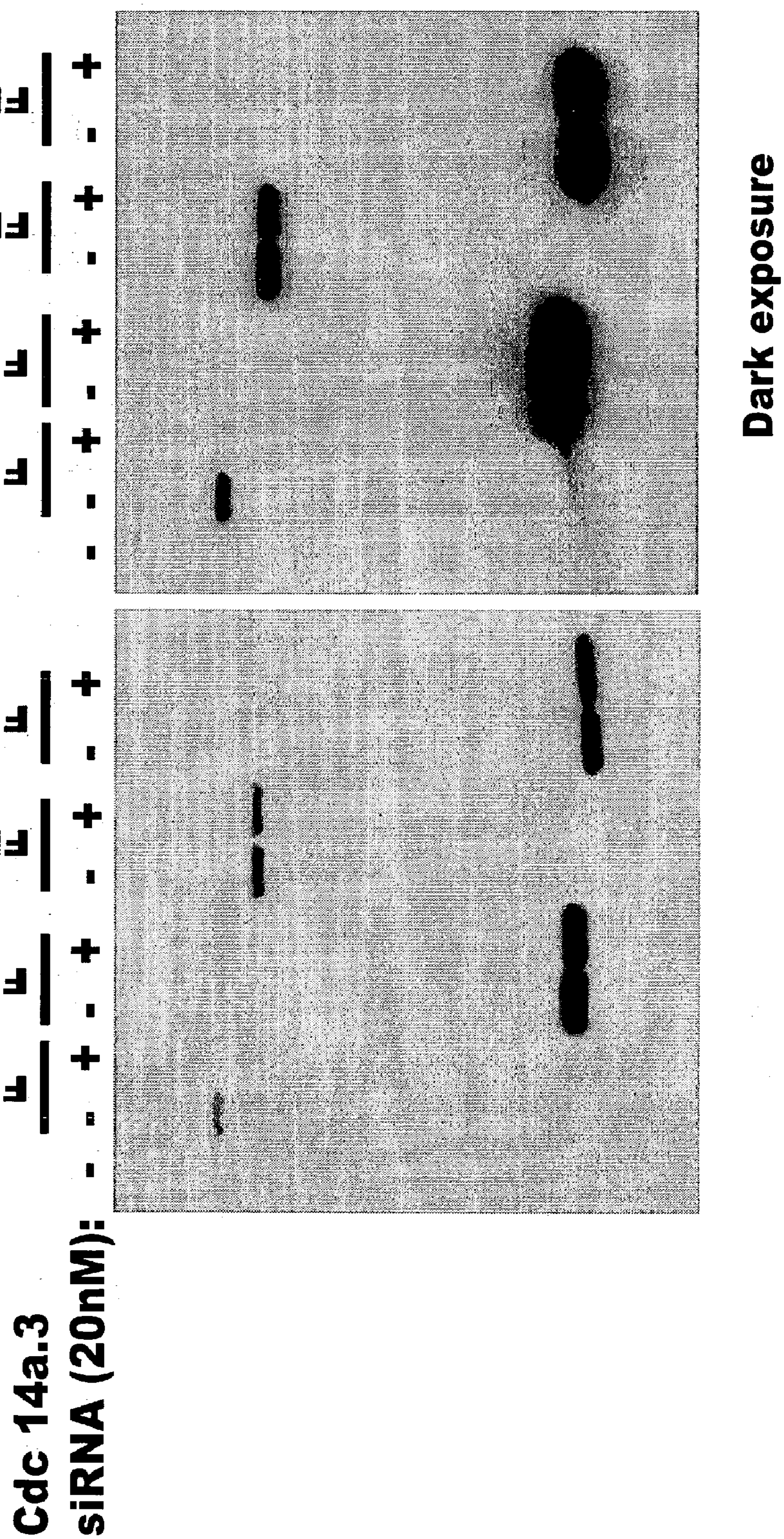

Cdc 14a.3
siRNA (20nM):
Flag 14a
Flag DSP3
Flag 14b
Flag DSP11
Dark exposure

Fig. 7

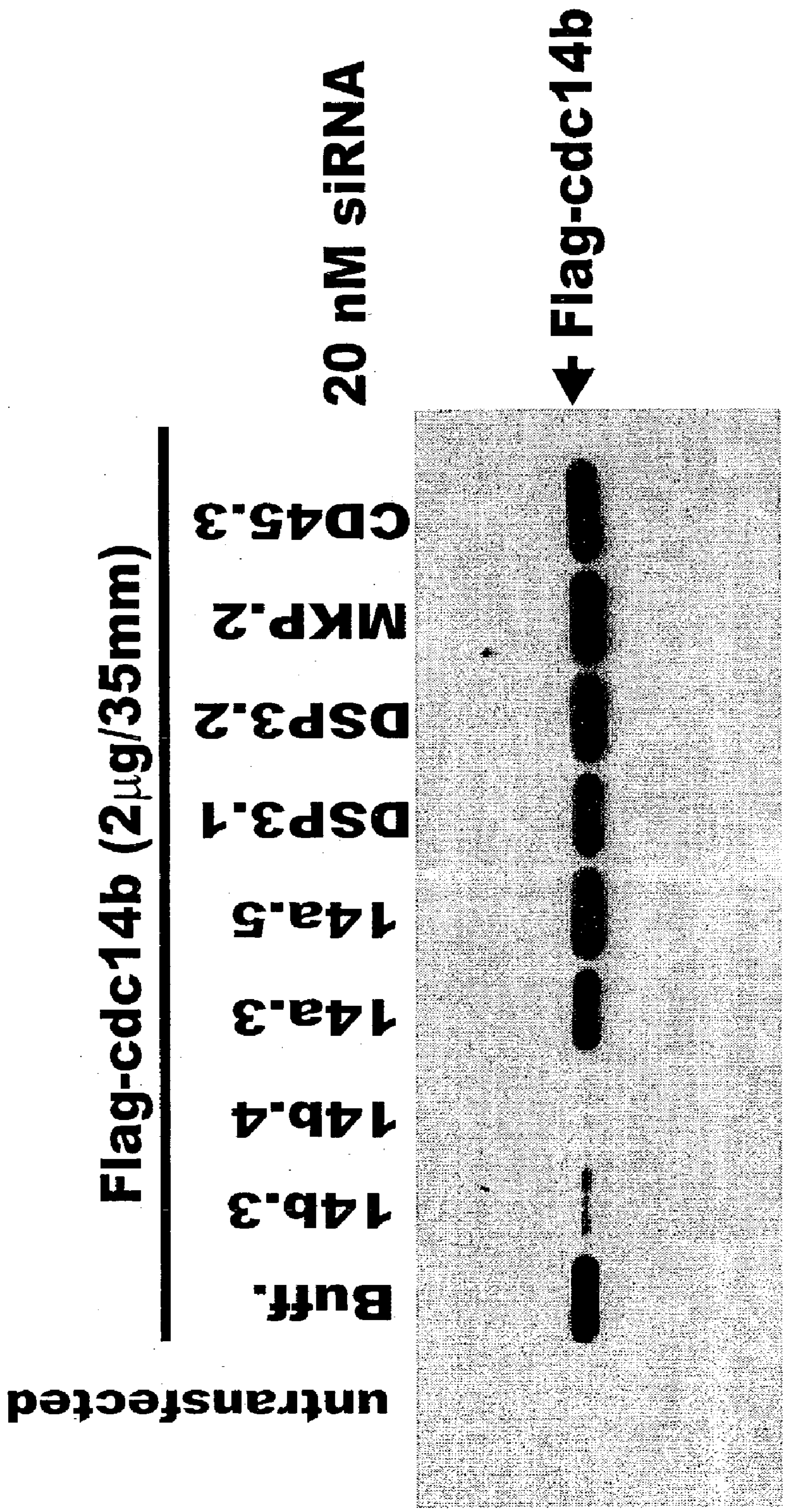
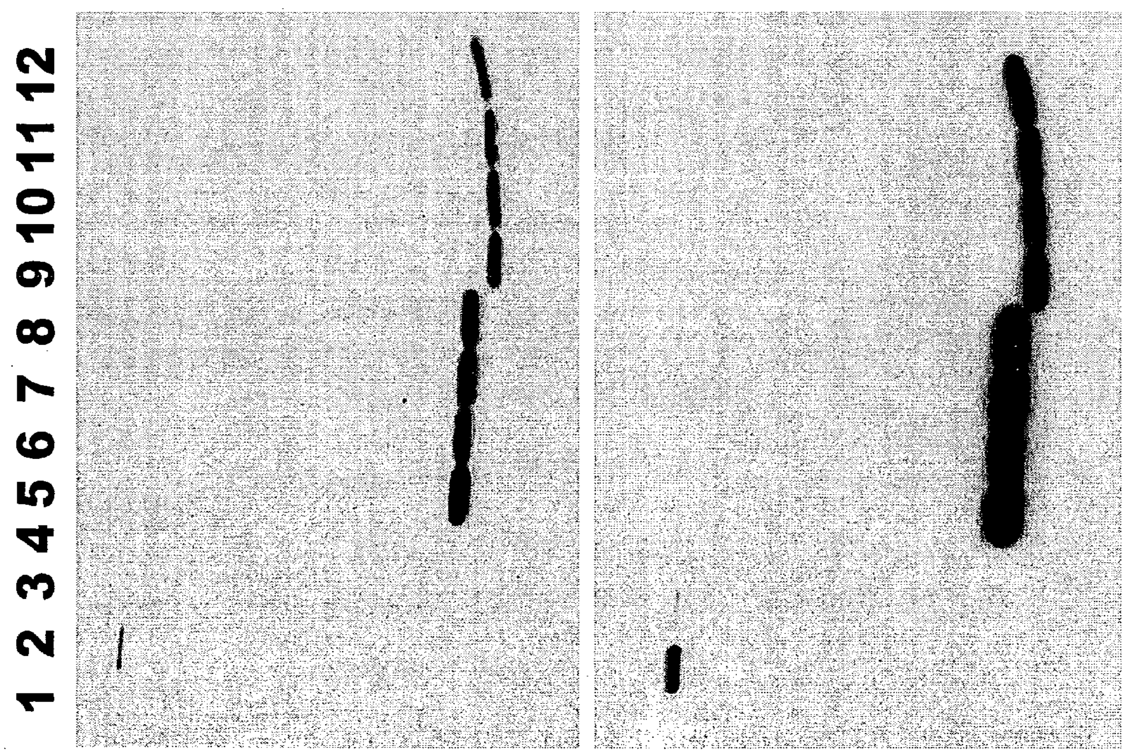
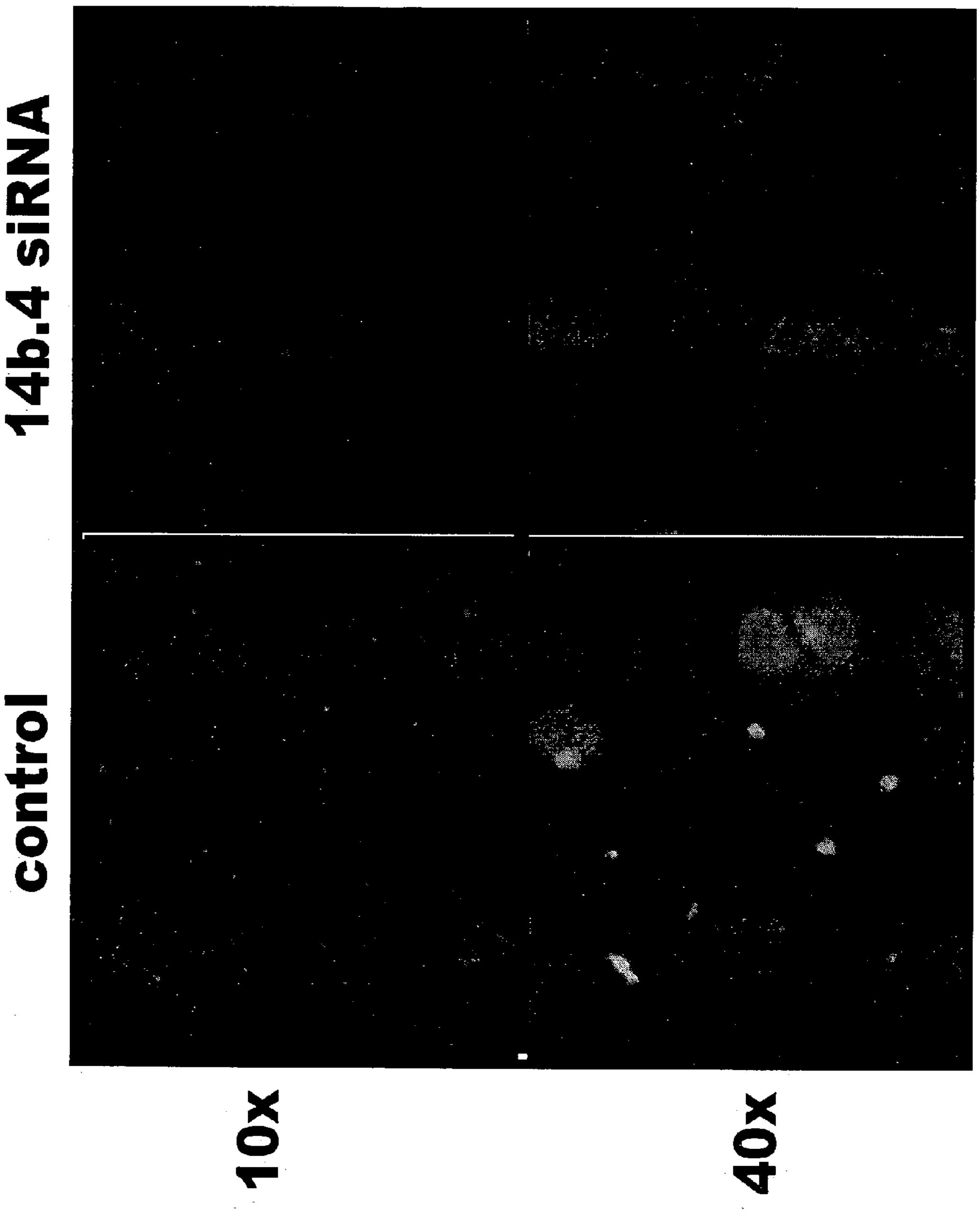

20 nM siRNA
Flag-cdc14b
Flag-cdc14b (2μg/35mm)
untransfected
Buff.
14b.3
14b.4
14a.3
14a.5
DSP3.1
DSP3.2
MKP.2
CD45.3

Fig. 8



1. Non-transfected
2. Flag-cdc14b
3. Flag-cdc14b + 14b.3 siRNA
4. Flag-cdc14b + 14b.4 siRNA
5. Flag-DSP3
6. Flag-DSP3 + 14b.3 siRNA
7. Flag-DSP3 + 14b.4 siRNA
8. Flag-DSP3 + 14a.5 siRNA
9. Flag-DSP11
10. Flag-DSP11 + 14b.3 siRNA
11. Flag-DSP11 + 14b.4 siRNA
12. Flag-DSP11 + 14a.5 siRNA

Fig. 9



Anti cdc14b IF

Fig. 10

- Mouse fibroblasts were transfected with 200 nM RNAi oligonucleotides for a total of six days.
- "NT" is non-transfected fibroblasts.

Fig. 11

Fig. 12A

Prototypical DSP-18pr encoded by 708 base pairs

GGCCCCCCGTTCCCCGCCAGGCTGCAGGCGTCGGGCCTGGGCCGTCAGGGCAGCTGTGACCGGATCGCTTC
CCGGGCGGCGAGCTGGGGGTGCACCCGGACCGCCGCCCCCGGGATC**ATG**GGCAATGGCATGACCA**AGGT**AC
TTCCTGGACTCTACCTCGGAAACTTCAT**TGAT**GCCAAAGACCTGGATCAGCTGGGCCGAAATAAGATCACA
CACATCATCTCTATCCATGAGTCACCCCAGCCTCTGCTGC**AGGA**TATCACCTACCTTCGCATCCCGGTCGC
TGATACCCCTGAGGTACCC**ATCA**AAAAGCACTTCAAAGAATGTATCAACTTCATCCACTGCTGCCGCCTTA
ATGGGGGGAACTGCCTTGTGCAC**TGCT**TTGCAGGCATCTCTCGCAGCACCACGATTGTGACAGCGTATGTG
ATGACTGTGACGGGGCTAGGCTGGCGGGACGTGCTTGAAGCCATCAAGGCCACCAGGCCCATCGCCAACCC
CAACCCAGGCTTTAGGCAGCAGCTTGAAGAGTTTGGCTGGGCCAGTTCCCAGA**AGCT**TCGCCGGCAGCTGG
AGGAGCGCTTCGGCGAGAGCCCCTTCCGCGACGAGGAGGAGTTGCGCGCGCTGCTGCCGCTGTGCAAGCGC
TGCCGGCAGGGCTCCGCGACCTCGGCCTCCTCCGCCGGGCCGCACTCAGCAGCCTCCGAGGGAACCGTGCA
GCGCCTGGTGCCGCGCACGCCCCGGGAAGCCCACCGGCCGCTGCCGCTGCTGGCGCGCGTCAAGCAGACTT
TCTCTTGCCTCCCCCGGTGTCTGTCCCGCAAGGGCGGCAAG**TGA**GGATGCAG

Fig. 12B

Prototypical DSP-18pr polypeptide sequence 235 amino acids

MGNGMTKVLPGLYLGNFIDAKDLDQLGRNKITHIISIHESPQPLLQDITYLRIPVADTPEVPIKKHFKECI
NFIHCCRLNGGN**CLVHCFAGISRSTTIVTAYVM**TVTGLGWRDVLEAIKATRPIANPNPGFRQQLEEFGWAS
SQKLRRQLEERFGESPFRDEEELRALLPLCKRCRQGSATSASSAGPHSAASEGTVQRLVPRTPREAHRPLP
LLARVKQTFSCLPRCLSRKGGK*

Fig. 13A

DSP-18a cDNA

GGCCCCCCGTTCCCCGCCAGGCTGCAGGCGTCGGGCCTGGGCCGTCAGGGCAGCTGTGACCGGATCGCTTC
CCGGGCGGCGAGCTGGGGGTGCACCCGGACCGCCGCCCCCGGGATC**ATG**GGCAATGGCATGACCA**AGGT**AC
TTCCTGGACTCTACCTCGGAAACTTCATT**GAT**GCCAAAGACCTGGATCAGCTGGGCCGAAATAAGATCACA
CACATCATCTCTATCCATGAGTCACCCCAGCCTCTGCTGC**AGGA**TATCACCTACCTTCGCATCCCGGTCGC
TGATACCCCTGAGGTACCC**ATCA**AAAAGCACTTCAAAGAATGTATCAACTTCATCCACTGCTGCCGCCTTA
ATGGGGGAACTGCCTTGTGCAC**TGCT**TTGCAGGCATCTCTCGCAGCACCACGATTGTGACAGCGTATGTG
ATGACTGTGACGGGGCTAGGCTGGCGGGACGTGCTTGAAGCCATCAAGGCCACCAGGCCCATCGCCAACCC
CAACCCAGGCTTTAGGCAGCAGCTTGAAGAGTTTGGCTGGGCCAGTTCCCAGA**AGGG**TGCCAGACATAGGA
CCTCAAAAACCTCTGGTGCCCAATGCCCTCCGATGACTTCAGCAACCT**GGA**TGGTCACCGGACCCAAAGTA
CCAGATCTGTCTGTGCTTCGGT**GA**GGAGGACCCGGGCCCCACACAGCACCCCAAGGAGCAGCTCATCATGG
CGG**ACGT**GCAGGTGCAGCTTCGGCCTGGGAGCTCGTCCTGCACTCTAAGTGCCTCAACCGAGCGCCCAGAT
GGGTCCTCAACCCCTGGCAACCCCGATGGCATCACTCACCTTCAATGCAGCTGCCTCCATCCTAAGCGAGC
CGCTTCCTCTTCTTGTACCCGCTGAAGGCAGCCCCCAACAGGGGGGCTCCCTACTCCCACCCAACCCTGCC
CACACTAAGCCCATAGACTTGGGGCCTCCCCCGGCACATCACCCAGGTCTGCCGGACGGCAGAGGTGGATC
GCGGCCTTCCACTCCTCTGTCACGGGGCCCCGGAACTCGAGAGTAGGCCACACCGCCCCCCAGCTGGGCAT
GGGGCTTCGGCAGGAAACTGAACTTGATCTTGAGGCCCCAGAAAGGCAGCAACTGGAGCAGAAGCAAGACT
TCATCTCTTGCTGACAGCCCAATTTGTCAATAGCGCTTTCCTCAGAGCCAGCCTTAACCTGCTGTTGAGTC
CATTAAAACGTTTGCTTAAAGTTTTTACCAATAATTAGATCATCAGGGTTGTTTAGTGTGGGATCAAGCCA
TAACAAAACTGCCTAGCCTCTCAGGGGCCTAGAATTTACAGAACCTTCCTCCTCCCTGCAGCAAGTCTCTC
TTCTTTATTCTGGGGGCTGGGAAGGATCCCAAAACAGGGAACTTGGCCGAACCCTGGGCTTTGGATGCTAA
CCACTGAAGTACCAGCACCTGTAGGATGCTGTCTTTGAAGAAACTGAGGCGGACCTCCAAATGCAGCCCTA
AGGCAGAGGTCAACGTGGAAGACCAGCCCTTCTCCAAGCCCCACTGGTCTTTGCAAGCTGTACGTTGTAGG
CAATCTGAGAACTGGAAAGGGGGACTACAACCAGAAAGTTGGTTACCCTGCCATGGGAATAAAGTAGCTGT
TTTCCACCCCAAAAAAAAAAAAAAAAAAAAAA

Fig. 13B

DSP-18a polypeptide (181 amino acids)

MGNGMTKVLPGLYLGNFIDAKDLDQLGRNKITHIISIHESPQPLLQDITYLRIPVADTPEVPIKKHFKECI
NFIHCCRLNGGN**CLVHCFAGISRSTTIVTAYVM**TVTGLGWRDVLEAIKATRPIANPNPGFRQQLEEFGWAS
SQKGARHRTSKTSGAQCPPMTSATWMVTGPKVPDLSVLR*

Fig. 14A

DSP-18b cDNA

GGCCCCCCGTTCCCCGCCAGGCTGCAGGCGTCGGGCCTGGGCCGTCAGGGCAGCTGTGACCGGATCGCTTC
CCGGGCGGCGAGCTGGGGGTGCACCCGGACCGCCGCCCCCGGGATC**ATG**GGCAATGGCATGACCA**AGGT**AC
TTCCTGGACTCTACCTCGGAAACTTCAT**TGAT**GCCAAAGACCTGGATCAGCTGGGCCGAAATAAGATCACA
CACATCATCTCTATCCATGAGTCACCCCAGCCTCTGCTGC**AGGA**TATCACCTACCTTCGCATCCCGGTCGC
TGATACCCCTGAGGTACCC**ATCA**AAAAGCACTTCAAAGAATGTATCAACTTCATCCACTGCTGCCGCCTTA
ATGGGGGGAACTGCCTTGTGCACT**GCTT**TGCAGGCATCTCTCGCAGCACCACGATTGTGACAGCGTATGTG
ATGACTGTGACGGGGCTAGGCTGGCGGGACGTGCTTGAAGCCATCAAGGCCACCAGGCCCATCGCCAACCC
CAACCCAGGCTTTAGGCAGCAGCTTGAAGAGTTTGGCTGGGCCAGTTCCCAGA**AGGG**TGCCAGACATAGGA
CCTCAAAAACCTCTGGTGCCCAATGCCCTCCGATGACTTCAGCAACC**TGCC**TGCTGGCTGCACGTGTGGCT
CTTCTCTCCGCAGCGCTGGTGCGCGAAGCCACCGGGCGCACAGCCCAGCGCTGTCGTCTGAGTCCGCGGGC
GGCCGCCGAGCGCCTGCTGGGGCCGCCACCTCACGTTGCAGC**AGGA**TGGTCACCGGACCCAAAGTACCAGA
TCTGTCTGTGCTTCGGTGAGGAGGACCCGGGCCCCACACAGCACCCCAAGGAGCAGCTCATCATGGCGG**AC**
GTGCAGGTGCAGCTTCGGCCTGGGAGCTCGTCCTGCACTCTAAGTGCCTCAACCGAGCGCCCAGATGGGTC
CTCAACCCCTGGCAACCCCGATGGCATCACTCACCTTCAATGCAGCTGCCTCCATCCTAAGCGAGCCGCTT
CCTCTTCTTGTACCCGC**TGA**AGGCAGCCCCCAACAGGGGGGCTCCCTACTCCCACCCAACCCTGCCCACAC
TAAGCCCATAGACTTGGGGCCTCCCCGGCGGCACATCACCCAGGTCTGCCGGACGGCAGAGGTGGATCGCG
GCCTTCCACTCCTCTGTCACGGGGCCCCGGAACTCGAGAGTAGGCCACACCGCCCCCCAGCTGGGCATGGG
GCTTCGGCAGGAAACTGAACTTGATCTTGAGGCCCCAGAAAGGCAGCAACTGGAGCAGAAGCAAGACTTCA
TCTCTTGCTGACAGCCCAATTTGTCAATAGCGCTTTCCTCAGAGCCAGCCTTAACCTGCTGTTGAGTCCAT
TAAAACGTTTGCTTAAAGTTTTTACCAATAATTAGATCATCAGGGTTGTTTAGTGTGGGATCAAGCCATAA
CAAAACTGCCTAGCCTCTCAGGGGCCTAGAATTTACAGAACCTTCCTCCTCCCTGCAGCTAGTCTCTCTTC
TTTATTCTGGGGGCTGGGAAGGATCCCAAAACAGGGAACTTGGCCGAACCCTGGGCTTTGGATGCTAACCA
CTGAAGTACCAGCACCTGTAGGATGCTGTCTTTGAAGAAACTGAGGCGGACCTCCAAATGCAGCCCTAAGG
CAGAGGTCAACGTGGAAGACCAGCCCTTCTCCAAGCCCCACTGGTCTTTGCAAGCTGTACGTTGTAGGCAA
TCTGAGAACTGGAAAGGGGGACTACAACCAGAAAGTTGGTTACCCTGCCATGGGAATAAAGTAGCTGTTTT
CCACCCCAAAAAAAAAAAAAAAAAAAAAAAAA

Fig. 14B

DSP-18b polypeptide (298 amino acids)

MGNGMTKVLPGLYLGNFIDAKDLDQLGRNKITHIISIHESPQPLLQDITYLRIPVADTPEVPIKKHFKECI
NFIHCCRLNGGN**CLVHCFAGISRSTTIVTAYVM**TVTGLGWRDVLEAIKATRPIANPNPGFRQQLEEFGWAS
SQKGARHRTSKTSGAQCPPMTSATCLLAARVALLSAALVREATGRTAQRCRLSPRAAAERLLGPPPHVAAG
WSPDPKYQICLCFGEEDPGPTQHPKEQLIMADVQVQLRPGSSSCTLSASTERPDGSSTPGNPDGITHLQCS
CLHPKRAASSSCTR*

Fig. 15A

DSP-18c cDNA

GGCCCCCCGTTCCCCGCCAGGCTGCAGGCGTCGGGCCTGGGCCGTCAGGGCAGCTGTGACCGGAT
CGCTTCCCGGGCGGCGAGCTGGGGGTGCACCCGGACCGCCGCCCCCGGGATC**ATG**GGCAATGGCA
TGACCA**AGGT**ACTTCCTGGACTCTACCTCGGAAACTTCAT**TGAT**GCCAAAGACCTGGATCAGCTG
GGCCGAAATAAGATCACACACATCATCTCTATCCATGAGTCACCCCAGCCTCTGCTGC**AGGA**TAT
CACCTACCTTCGCATCCCGGTCGCTGATACCCCTGAGGTACCC**ATCA**AAAAGCACTTCAAAGAAT
GTATCAACTTCATCCACTGCTGCCGCCTTAATGGGGGGAACTGCCTTGTGCAC**TGCT**TTGCAGGC
ATCTCTCGCAGCACCACGATTGTGACAGCGTATGTGATGACTGTGACGGGGCTAGGCTGGCGGGA
CGTGCTTGAAGCCATCAAGGCCACCAGGCCCATCGCCAACCCCAACCCAGGCTTTAGGCAGCAGC
TTGAAGAGTTTGGCTGGGCCAGTTCCCAGA**AGGG**TGCCAGACATAGGACCTCAAAAACCTCTGGT
GCCCAATGCCCTCCGATGACTTCAGCAACC**TGGA**TGGTCACCGGACCCAAAGTACCAGATCTGTC
TGTGCTTCGGTGAGGAGGACCCGGGCCCCACACAGCACCCCAAGGAGCAGCTCATCATGGCGG**AC**
GTGCAGGTGCAGCTTCGGCCTGGGAGCTCGTCCTGCACTCTAAGTGCCTCAACCGAGCGCCCAGA
TGGGTCCTCAACCCCTGGCAACCCCGATGGCATCACTCACCTTCAATGCAGCTTGCCTCCATCCT
AAGCGAGCCGCTTCCTCTTCTTGTACCCGCTGAAGGCAAGCCCCCAACAGGGGGGCTCCCTACTC
CCACCCAACCCTGCCCACACTAAGCCCATAGACTTGGGGCCTCCCCCGGCACATCACCCAGGTCT
GCCGGACGGCAGAGGTGGATCGCGGCCTTCCACTCCTCTGTCACGGGGCCCCGGAACTCGAGAGT
AGGCCTCACCGCCCCCCAGCTGGGCATGGGGCTTCGGCAGGAAACTGAACTTGATCTTGAGGCCA
GCAGAAAGGCAGCAACTGGAGCAGAAGCAAGACTTCATCTCTTGCTGACAGCCCAATTTGTCAAT
AGCGCTTTCCTCAGAGCCAGCCTTAACCTGCTGTTGAGTCCATTAAAACGTTTGCTTAAAGTTTT
TACCAATAAAAAAAAAAAAAAAAAAAAAAAAA

Fig. 15B

DSP-18d cDNA

GGCCCCCCGTTCCCCGCCAGGCTGCAGGCGTCGGGCCTGGGCCGTCAGGGCAGCTGTGACCGGATCGCTTC
CCGGGCGGCGAGCTGGGGGTGCACCCGGACCGCCGCCCCCGGGATC**ATG**GGCAATGGCATGACCA**AGGT**AC
TTCCTGGACTCTACCTCGGAAACTTCAT**TGAT**GCCAAAGACCTGGATCAGCTGGGCCGAAATAAGATCACA
CACATCATCTCTATCCATGAGTCACCCCAGCCTCTGCTGC**AGGA**TATCACCTACCTTCGCATCCCGGTCGC
TGATACCCCTGAGGTACCC**ATCA**AAAAGCACTTCAAAGAATGTATCAACTTCATCCACTGCTGCCGCCTTA
ATGGGGGGAACTGCCTTGTGCAC**TGCT**TTGCAGGCATCTCTCGCAGCACCACGATTGTGACAGCGTATGTG
ATGACTGTGACGGGGCTAGGCTGGCGGGACGTGCTTGAAGCCATCAAGGCCACCAGGCCCATCGCCAACCC
CAACCCAGGCTTTAGGCAGCAGCTTGAAGAGTTTGGCTGGGCCAGTTCCCAGA**AGGG**TGCCAGACATAGGA
CCTCAAAAACCTCTGGTGCCCAATGCCCTCCGATGACTTCAGCAACCTGGATGGTCACCGGACCCAAAGTA
CCAGATCTGTCTGTGCTTCGGTGAGGAGGACCCGGGCCCCACACAGCACCCCAAGGAGCAGCTCATCATGG
CGGA**CCT**AGTCTCTCTTCTTTATTCTGGGGGCTGGGAAGGATCCCAAAACAGGGAACTTGGCCGAACCCTG
GGCTTTGGATGCTAACCACTGAAGTACCAGCACCTGTAGGATGCTGTCTTTGAAGAAACTGAGGCGGACCT
CCAAATGCAGCCCTAAGGCAGAGGTCAACGTGGAAGACCAGCCCTTCTCCAAGCCCCACTGGTCTTTGCAA
GCTGTACGTTGTAGGCAATCTGAGAACTGGAAAGGGGGACTACAACCAGAAAGTTGGTTACCCTGCCATGG
GAATAAAGTAGCTGTTTTCCACCCCATAAAAAAAAAAAAAAAAAAAAAAA

Fig. 16A

DSP-18e cDNA

GGCCCCCCGTTCCCCGCCAGGCTGCAGGCGTCGGGCCTGGGCCGTCAGGGCAGCTGTGACCGGATCGCTTC
CCGGGCGGCGAGCTGGGGGTGCACCCGGACCGCCGCCCCCGGGATC**ATG**GGCAATGGCATGACCA**AGGT**AC
TTCCTGGACTCTACCTCGGAAACTTCATT**GAT**GCCAAAGACCTGGATCAGCTGGGCCGAAATAAGATCACA
CACATCATCTCTATCCATGAGTCACCCCAGCCTCTGCTGC**AGGA**TATCACCTACCTTCGCATCCCGGTCGC
TGATACCCCTGAGGTACCC**ATCA**AAAAGCACTTCAAAGAATGTATCAACTTCATCCACTGCTGCCGCCTTA
ATGGGGGGAACTGCCTTGTGCAC**TGC**TTTGCAGGCATCTCTCGCAGCACCACGATTGTGACAGCGTATGTG
ATGACTGTGACGGGGCTAGGCTGGCGGGACGTGCTTGAAGCCATCAAGGCCACCAGGCCCATCGCCAACCC
CAACCCAGGCTTTAGGCAGCAGCTTAAGAGTTTGGCTGGGCCAGTTCCCAGA**AGGA**TGGTCACCGGACCCA
AAGTACCAGATCTGTCTGTGCTTCGG**TGA**GGAGGACCCGGGCCCCACACAGCACCCCAAGGAGCAGCTCAT
CATGGCGG**ACCT**AGTCTCTCTTCTTTATTCTGGGGGCTGGGAAGGATCCCAAAACAGGGAACTTGGCCGAA
CCCTGGGCTTTGGATGCTAACCACTGAAGTACCAGCACCTGTAGGATGCTGTCTTTGAAGAAACTGAGGCG
GACCTCCAAATGCAGCCCTAAGGCAGAGGTCAACGTGGAAGACCAGCCCTTCTCCAAGCCCCACTGGTCTT
TGCAAGCTGTACGTTGTAGGCAATCTGAGAACTGGAAAGGGGGACTACAACCAGAAAGTTGGTTACCCTGC
CATGGGAATAAAGTAGCTGTTTTCCACCCCCCAAAAAAAAAAAAAAAAAAAAAAAAAA

Fig. 16B

DSP-18e polypeptide (159 amino acids)

MGNGMTKVLPGLYLGNFIDAKDLDQLGRNKITHIISIHESPQPLLQDITYLRIPVADTPEVPIKKHFKECI
NFIHCCRLNGGN**CLVHCFAGISRSTTIVTAYVM**TVTGLGWRDVLEAIKATRPIANPNPGFRQQLKSLAGPV
PRRMVTGPKVPDLSVLR*

Fig. 17A

DSP-18f cDNA

GGCCCCCCGTTCCCCGCCAGGCTGCAGGCGTCGGGCCTGGGCCGTCAGGGCAGCTGTGACCGGATCGCTTC
CCGGGCGGCGAGCTGGGGGTGCACCCGGACCGCCGCCCCCGGGATC**ATG**GGCAATGGCATGACCA**AGGT**AC
TTCCTGGACTCTACCTCGGAAACTTCAT**TGAT**GCCAAAGACCTGGATCAGCTGGGCCGAAATAAGATCACA
CACATCATCTCTATCCATGAGTCACCCCAGCCTCTGCTGC**AGGA**TATCACCTACCTTCGCATCCCGGTCGC
TGATACCCCTGAGGTACCC**ATCA**AAAAGCACTTCAAAGAATGTATCAACTTCATCCACTGCTGCCGCCTTA
ATGGGGGGAACTGCCTTGTGCACT**GCTT**TGCAGGCATCTCTCGCAGCACCACGATTGTGACAGCGTATGTG
ATGACTGTGACGGGGCTAGGCTGGCGGGACGTGCTTGAAGCCATCAAGGCCACCAGGCCCATCGCCAACCC
CAACCCAGGCTTTAGGCAGCAGCTTGAAGAGTTTGGCTGGGCCAGTTCCCAGA**AGGG**CTTTTACCAACCTC
ATAAGCTGTTGT**GA**GAACCAATTGAGACACTGCAGGAAAGTGTTTAGCCAGGCCCAGCACTGATGAGCAGT
CGGATGGTCACCGGACCCAAAGTACCAGATCTGTCTGTGCTTCGGTGAGGAGGACCCGGGCCCCACACAGC
ACCCCAAGGAGCAGCTCATCATGGCGG**ACCT**AGTCTCTCTTCTTTATTCTGGGGGCTGGGAAGGATCCCAA
AACAGGGAACTTGGCCGAACCCTGGGCTTTGGATGCTAACCACTGAAGTACCAGCACCTGTAGGATGCTGT
CTTTGAAGAAACTGAGGCGGACCTCCAAATGCAGCCCTAAGGCAGAGGTCAACGTGGAAGACCAGCCCTTC
TCCAAGCCCCACTGGTCTTTGCAAGCTGTACGTTGTAGGCAATCTGAGAACTGGAAAGGGGGACTACAACC
AGAAAGTTGGTTACCCTGCCATGGGAATAAAGTAGCTGTTTTCCAAAAAAAAAAAAAAAAAAAAAAAA

Fig. 17B

DSP-18f polypeptide (154 amino acids)

MGNGMTKVLPGLYLGNFIDAKDLDQLGRNKITHIISIHESPQPLLQDITYLRIPVADTPEVPIKKHFKECI
NFIHCCRLNGGNC**LVHCFAGISRSTTIVTAYVM**TVTGLGWRDVLEAIKATRPIANPNPGFRQQLEEFGWAS
SQKGFYQPHKLL*

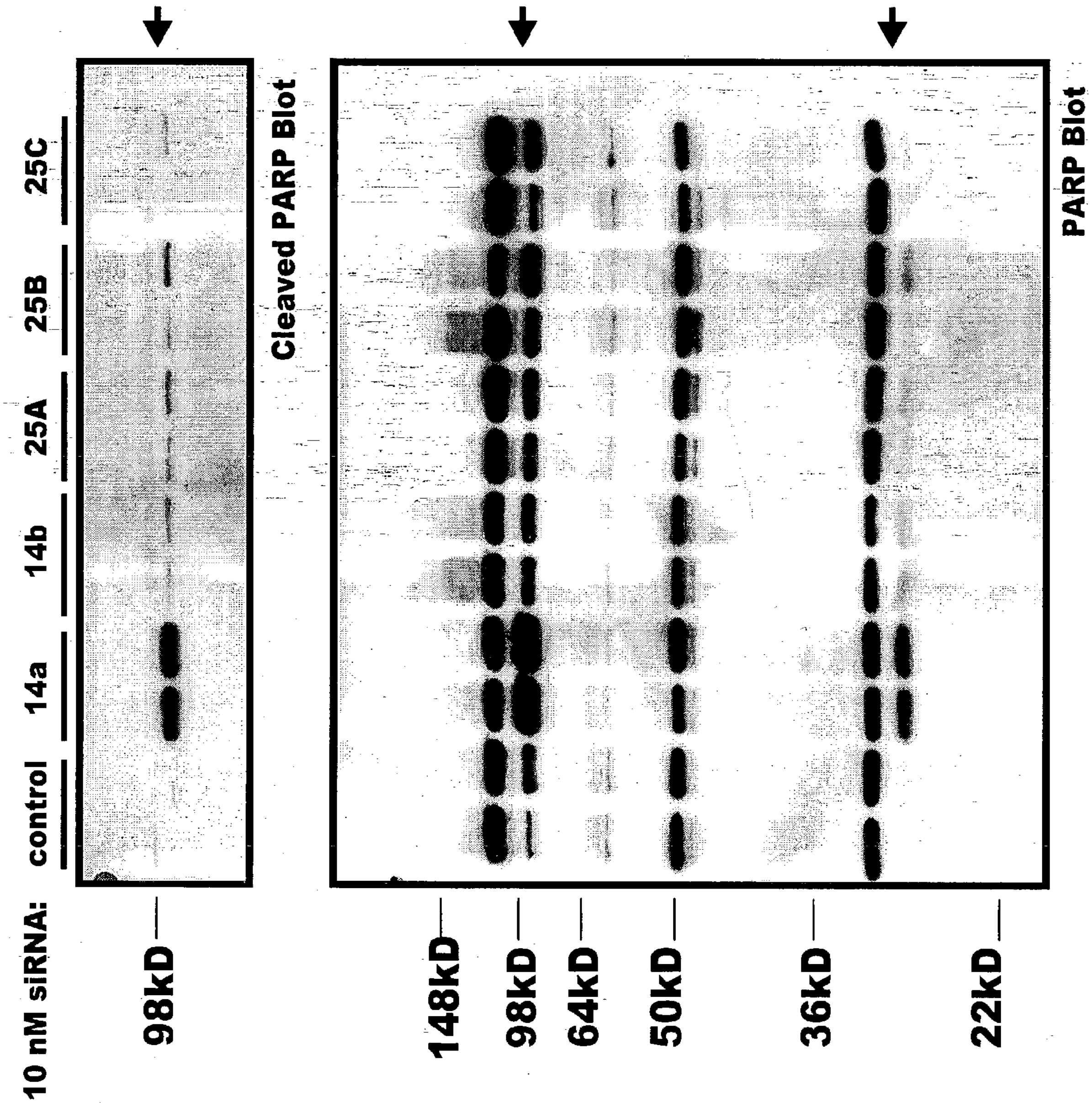

10 nM siRNA:
control
14a
14b
25A
25B
25C
98kD
Cleaved PARP Blot
148kD
98kD
64kD
50kD
36kD
22kD
PARP Blot

Fig. 18

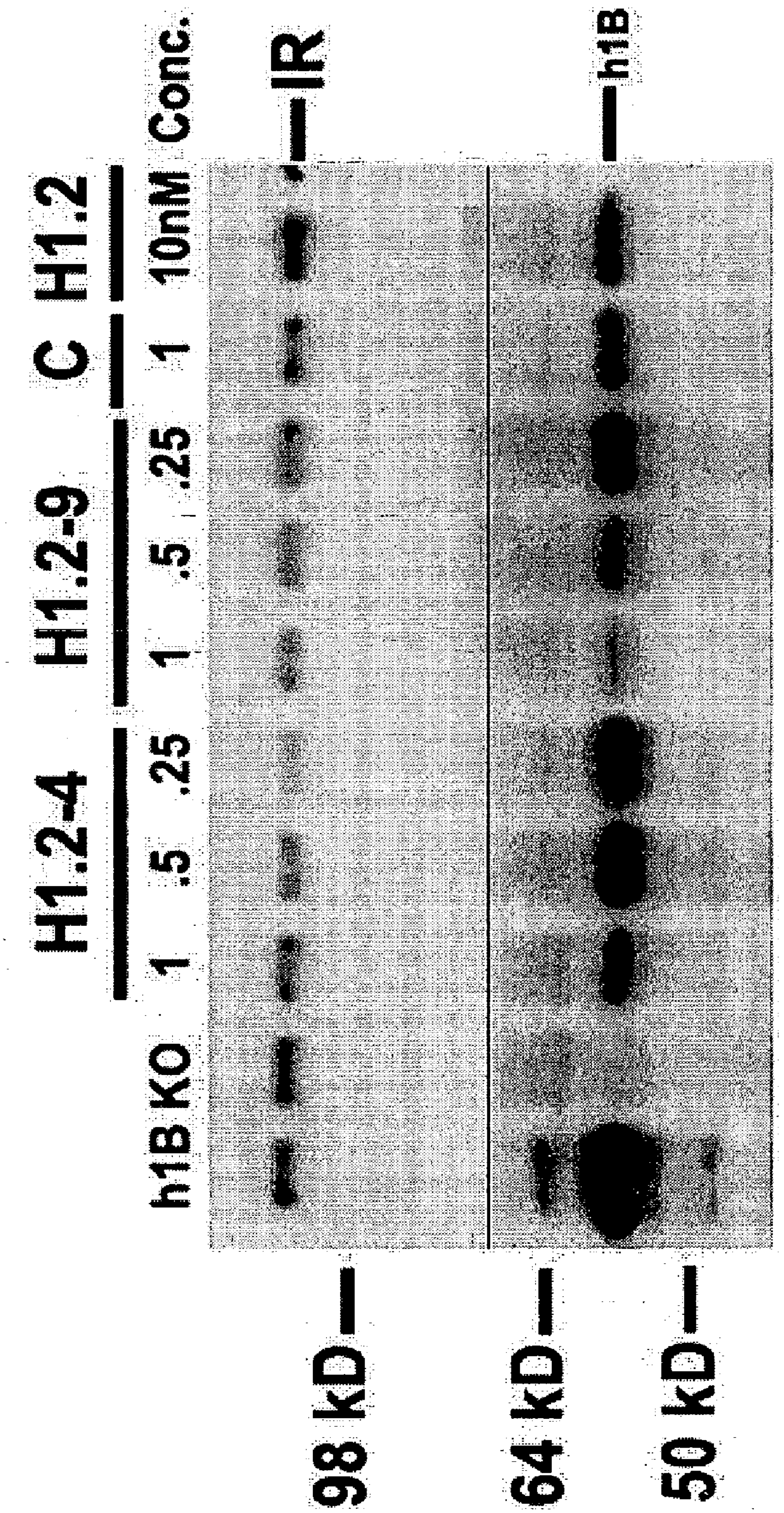

h1B
KO
H1.2-4
1
.5
.25
H1.2-9
1
.5
.25
C
1
H1.2
10nM
Conc.
IR
h1B
98 kD
64 kD
50 kD

Fig. 19

Fig. 20A

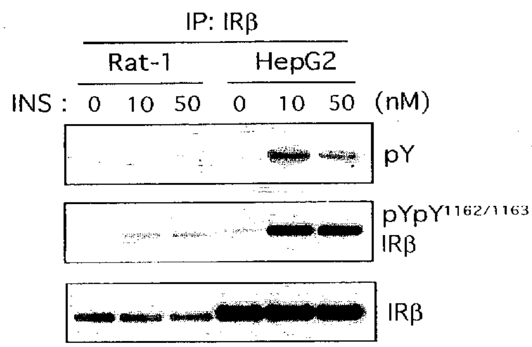


Fig. 20B

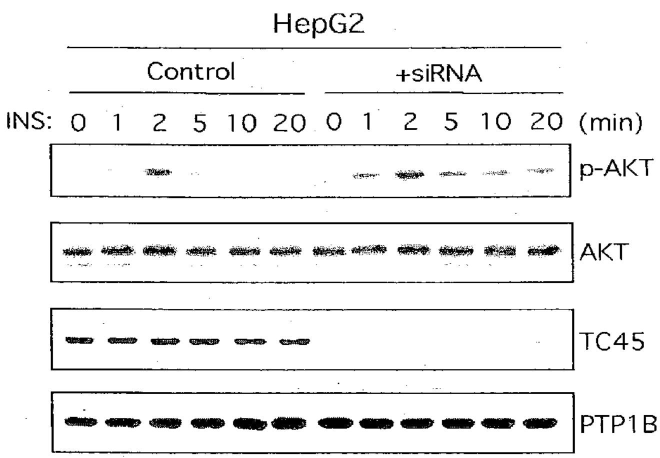


Fig. 20C

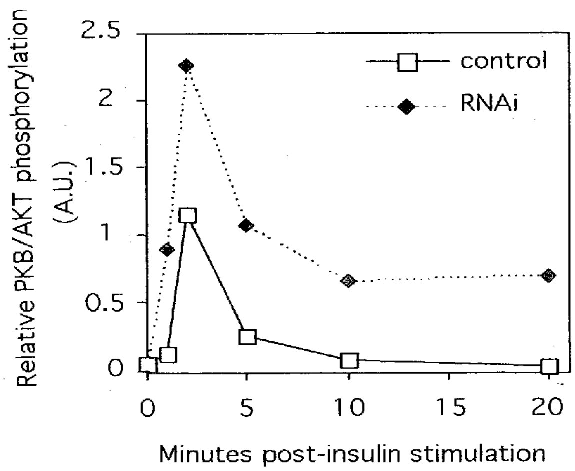


Fig. 21A

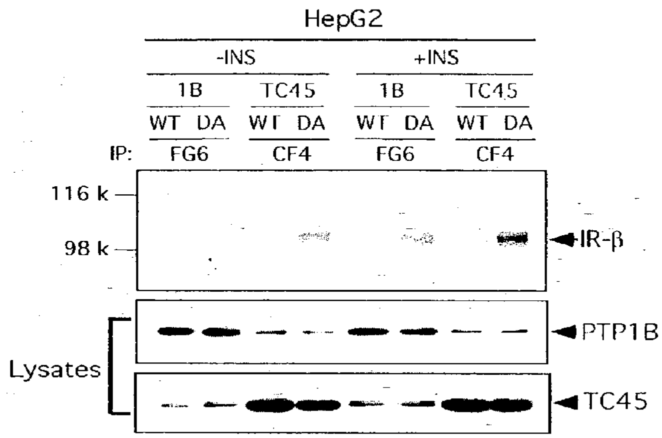


Fig. 21B

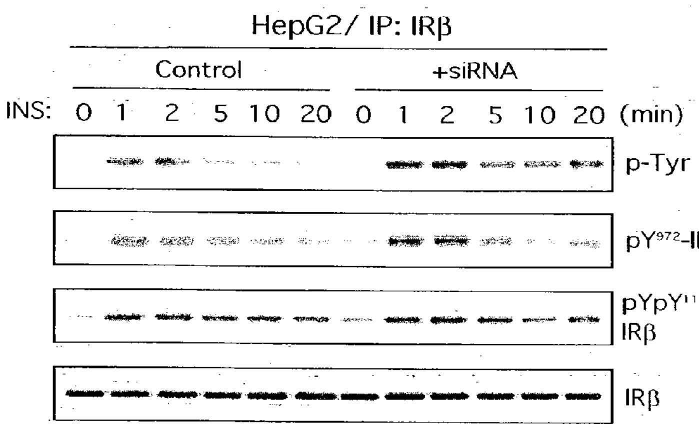


Fig. 21C

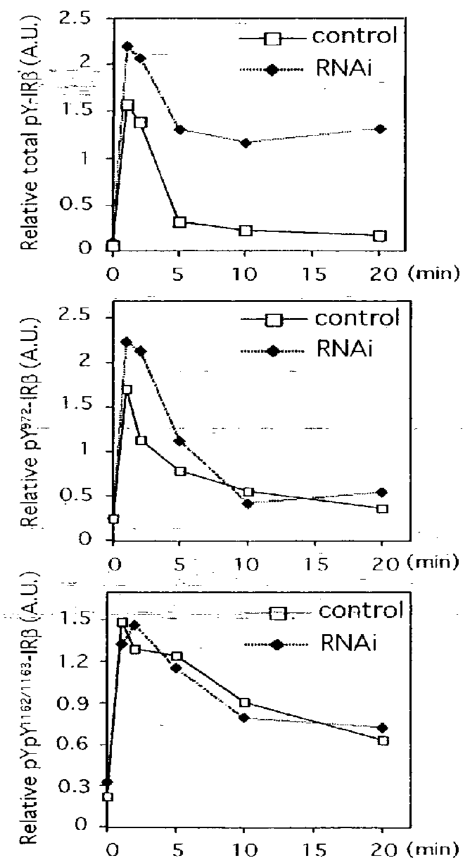
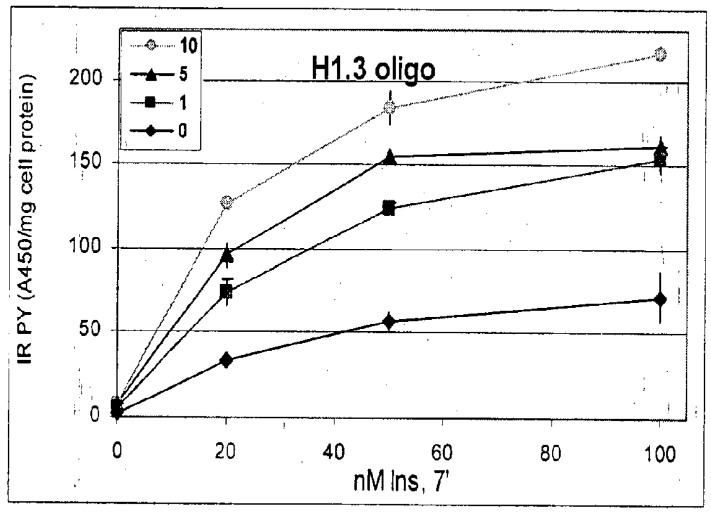
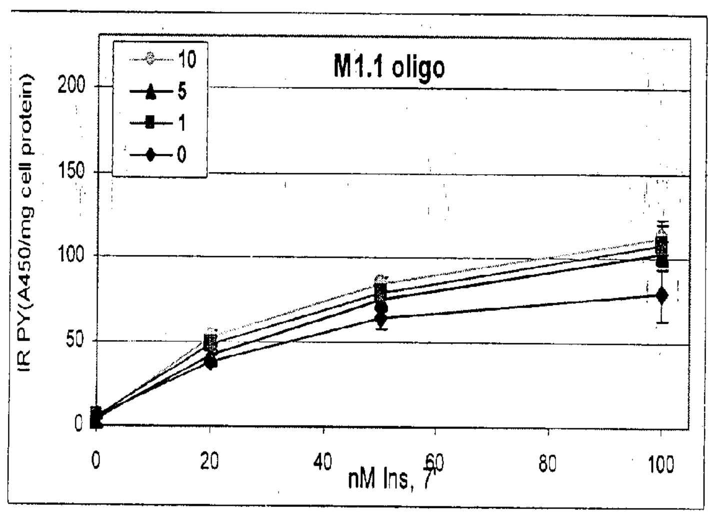


Fig. 22A



% PTP1B Remaining	
10 nM H1.3	8.9
5 nM H1.3	10.7
1 nM H1.3	22.4
0 nM H1.3	100.0

Fig. 22B



% PTP1B Remaining	
10 nM M1.1	112.9
5 nM M1.1	108.1
1 nM M1.1	135.0
0 nM M1.1	130.0

Fig. 23A

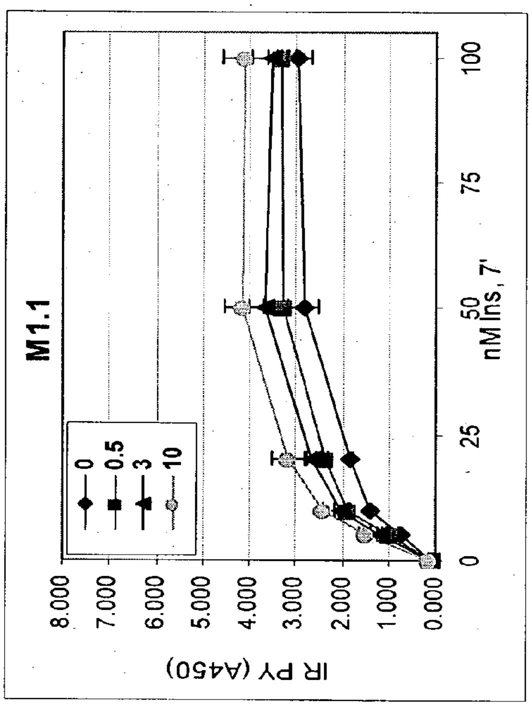


Fig. 23B

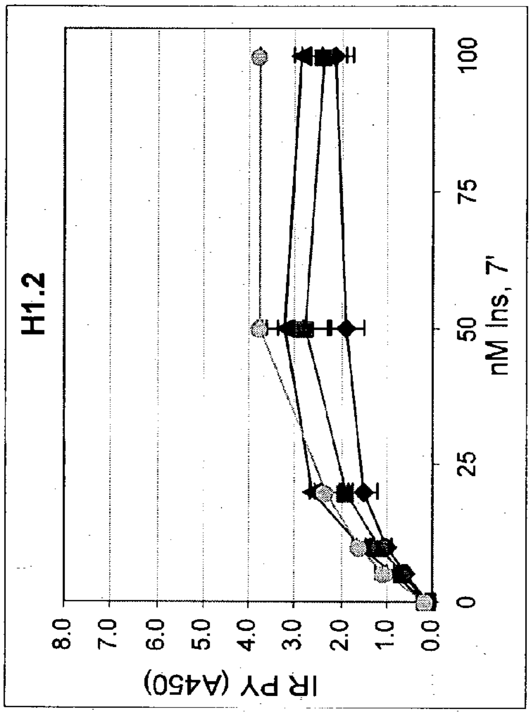


Fig. 23C

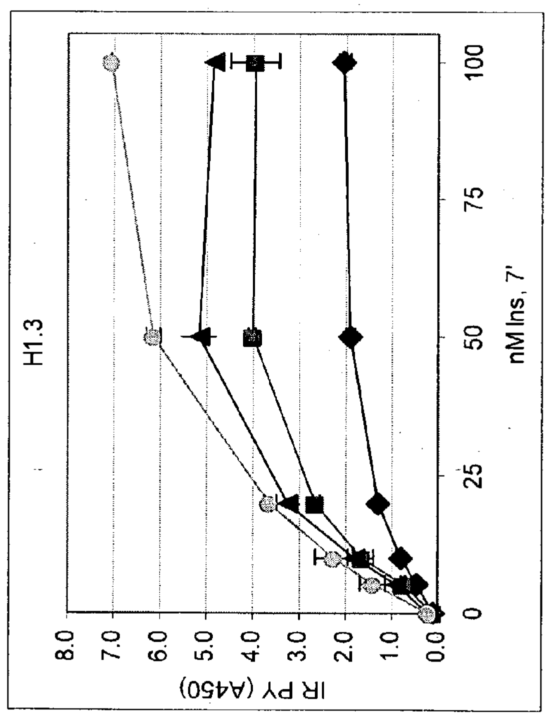
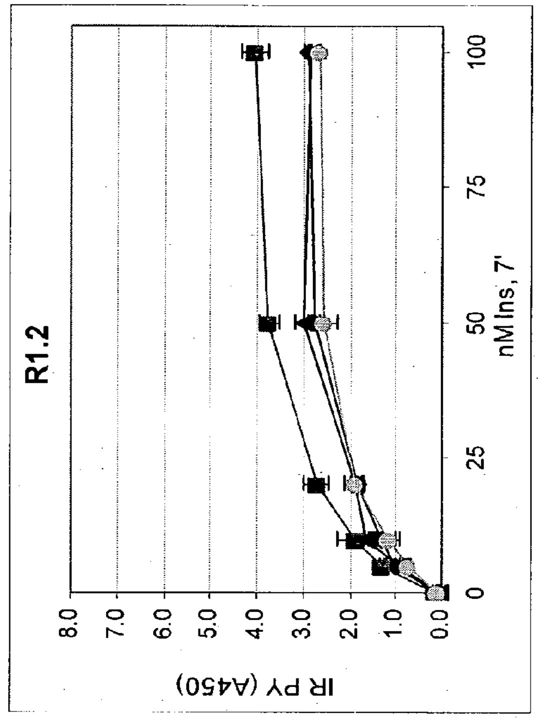


Fig. 23D



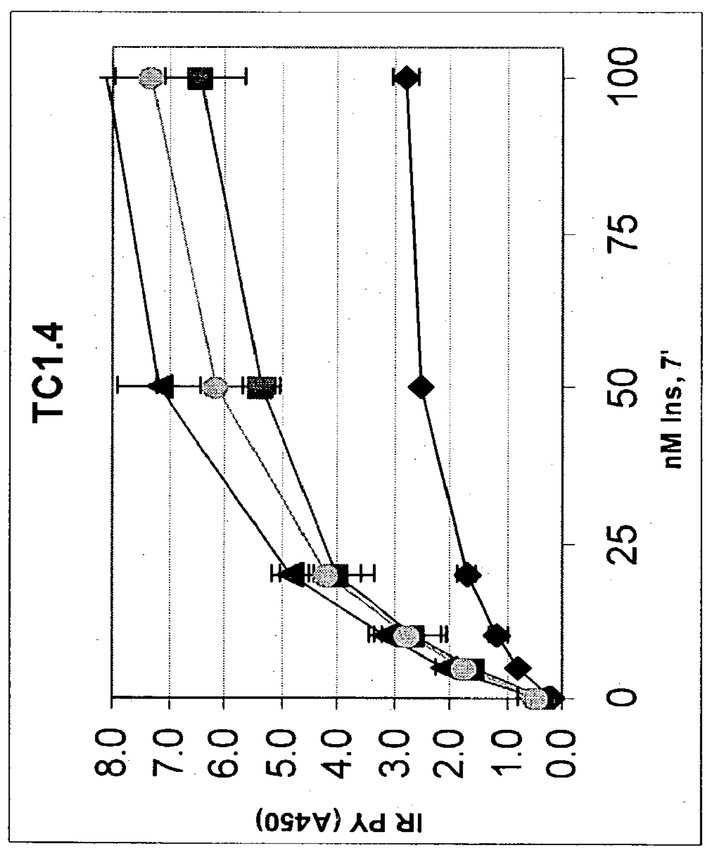


Fig. 24A

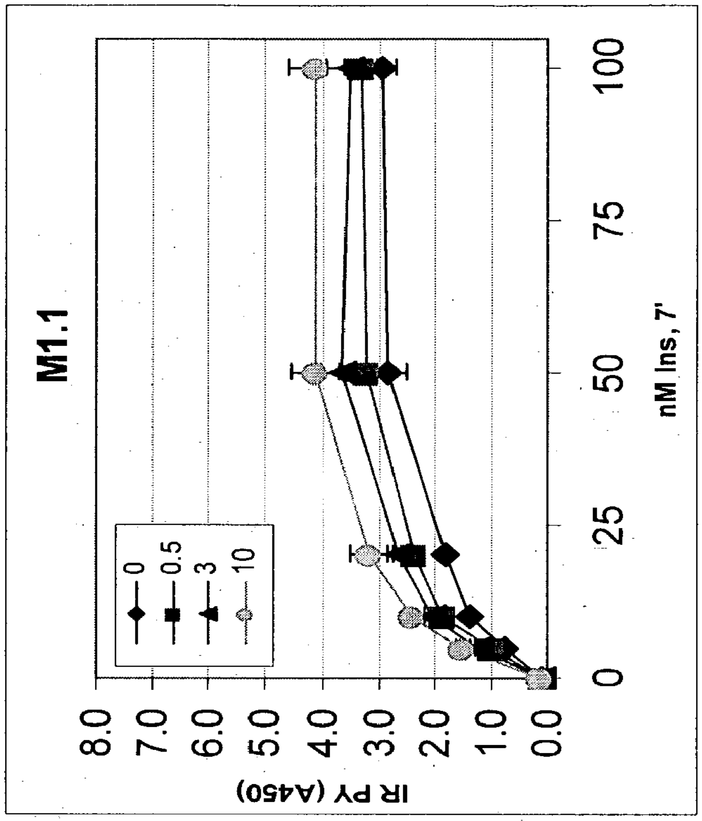
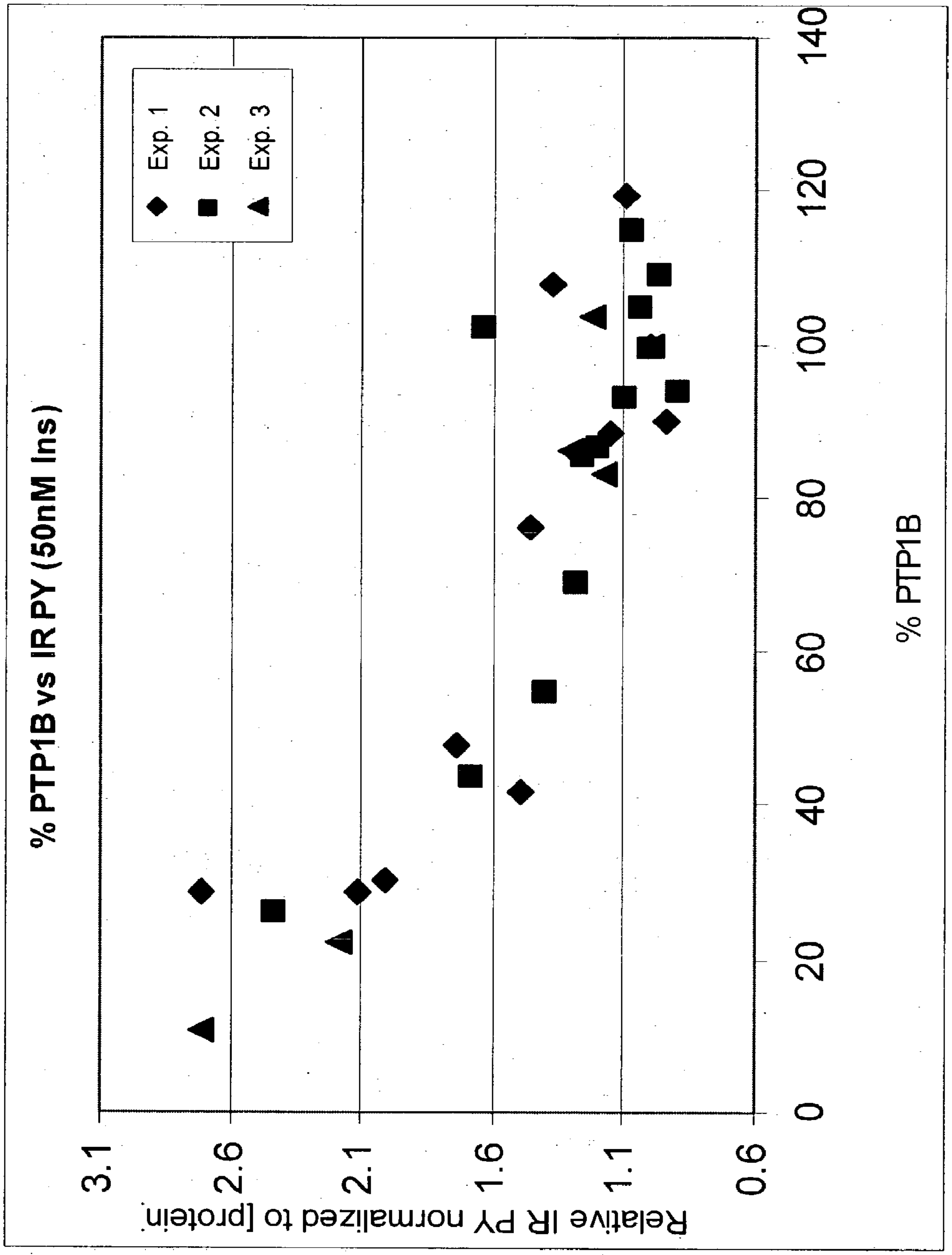


Fig. 24B



% PTP1B vs IR PY (50nM Ins)
Exp. 1
Exp. 2
Exp. 3
Relative IR PY normalized to [protein]
3.1
2.6
2.1
1.6
1.1
0.6
0
20
40
60
80
100
120
140
% PTP1B

Fig. 25

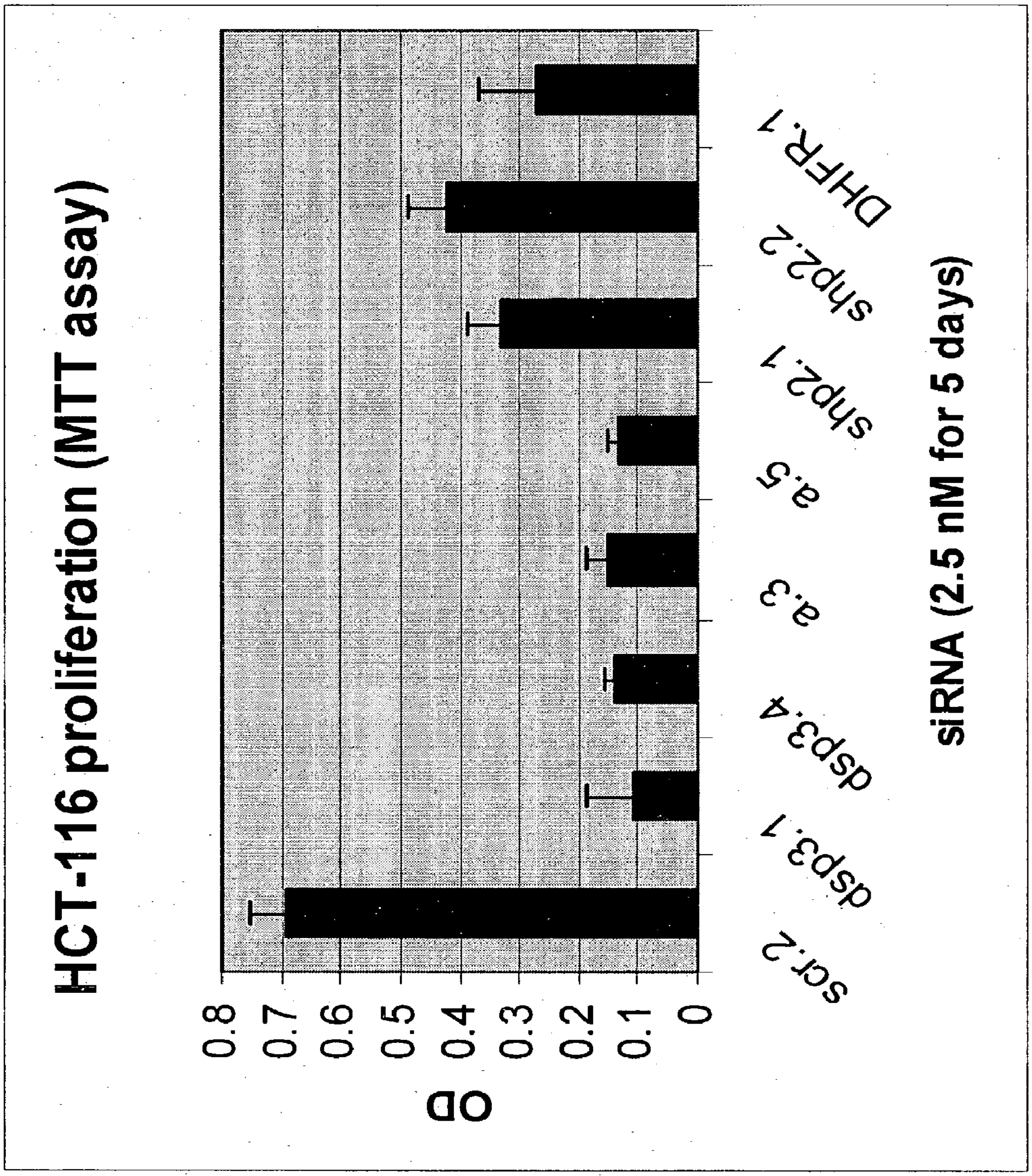

HCT-116 proliferation (MTT assay)
OD
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
scr.2
dsp3.1
dsp3.4
a.3
a.5
shp2.1
shp2.2
DHFR.1
siRNA (2.5 nM for 5 days)

Fig. 26

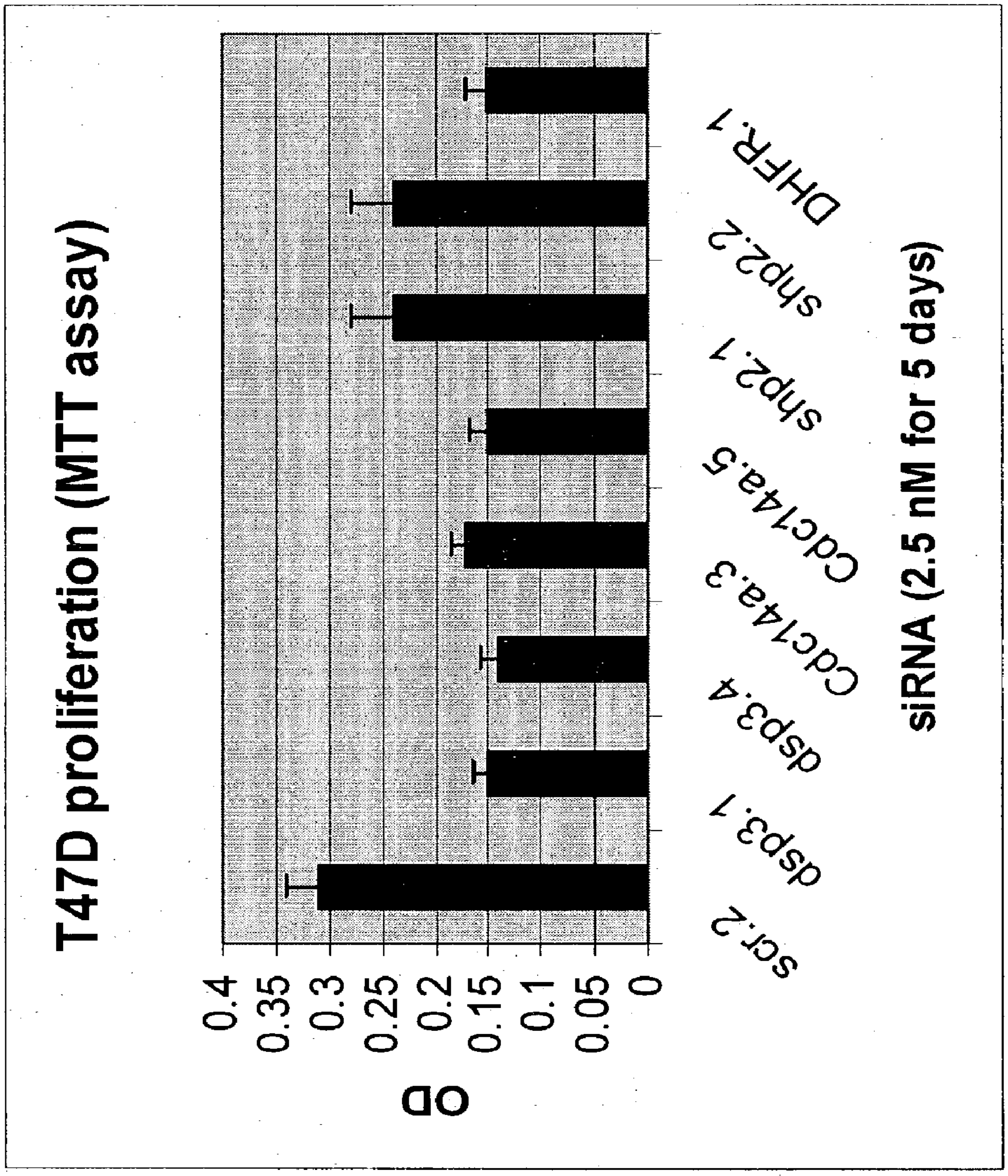

T47D proliferation (MTT assay)
OD
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
scr.2
dsp3.1
dsp3.4
Cdc14a.3
Cdc14a.5
shp2.1
shp2.2
DHFR.1
siRNA (2.5 nM for 5 days)

Fig. 27

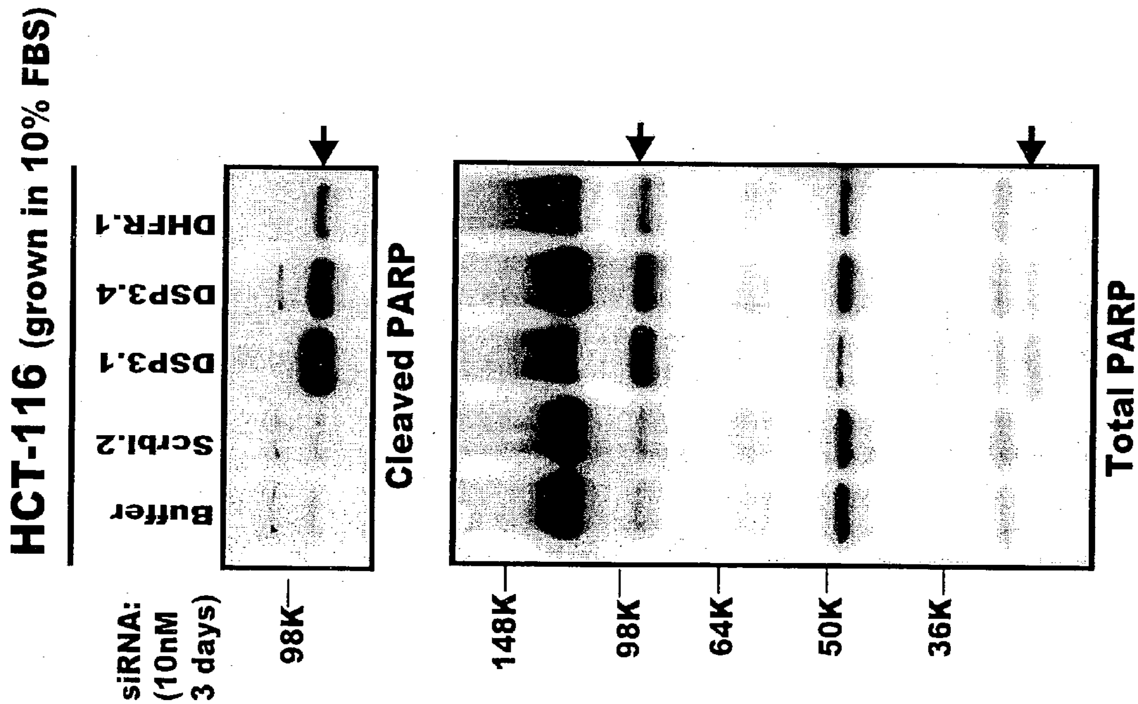


Fig. 28A

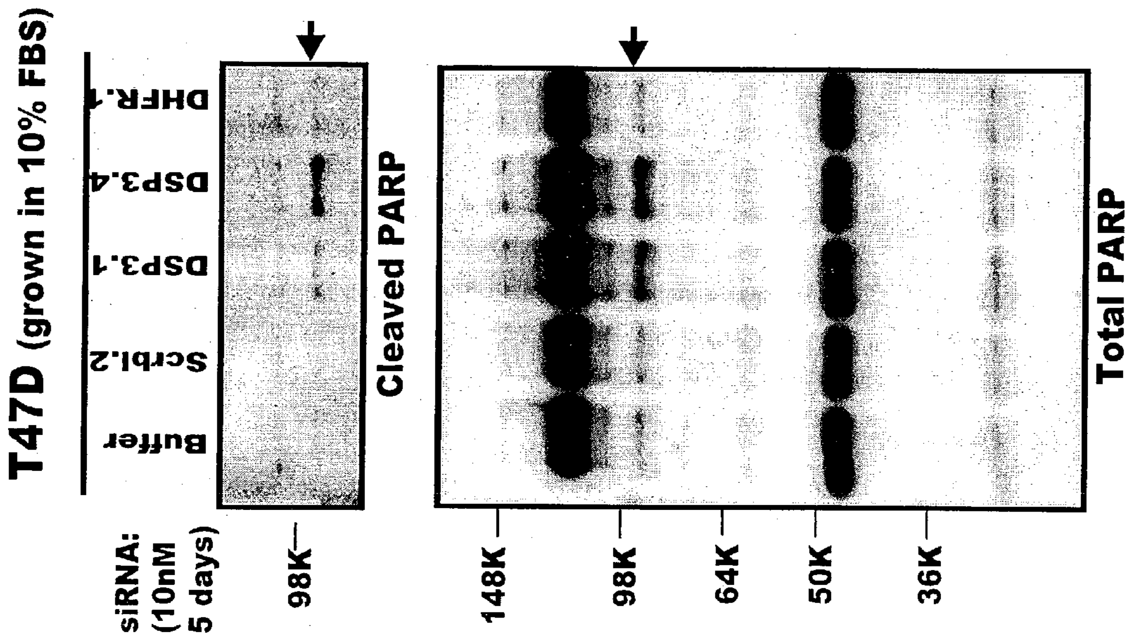


Fig. 28B

DSP-13 Encoding Polynucleotide

cctgggaaga agttatctat ctctcgagtg acattcaaga tataccgtac ccctcggttc 60
tgtaagtcct ctaagttgga ggcattccat tctgagccgg cccc**atg**acc ctgagcacgt 120
tggcccgcaa gaggaaggcg cccctcgctt gcacctgcag cctcggtggc cccgacatga 180
ttccttactt ctccgccaac gcggtcatct cgcagaacgc catcaaccag ctcatcagcg 240
agagctttct aactgtcaaa ggtgctgccc tttctacc acggggaaat ggctcatcca 300
caccaagaat cagccacaga cggaacaagc atgcaggcga tctccaacag catctccaag 360
caatgttcat tttactccgc ccagaagaca acatcaggct ggctgtaaga ctggaaagta 420
cttaccagaa tcgaacacgc tatatggtag tggtttcaac taatggtaga caagacactg 480
aagaaagcat cgtcctagga atggatttct cctctaatga cagtagcact tgtaccatgg 540
gcttagtttt gcctctctgg agcgacacgc taattcattt ggatggtgat ggtgggttca 600
gtgtatcgac ggataacaga gttcacatat tcaaacctgt atctgtgcag gcaatgtggt 660
ctgcactaca gagcttacac aaggcttgtg aagtcgccag agcgcataac tactacccag 720
gcagcctatt tctcacttgg gtgagttatt atgagagcca tatcaactca gatcaatcct 780
cagtcaatga atggaatgca atgcaagatg tacagtccca ccggcccgac tctccagctc 840
tcttcaccga catacctact gaacgtgaac gaacagaaag gctaattaaa accaaattaa 900
gggagatcat gatgcagaag gatttggaga atattacatc caaagagata agaacagagt 960
tggaaatgca aatggtgtgc aacttgcggg aattcaagga atttatagac aatgaaatga 1020
tagtgatcct tggtcaaatg gatagcccta cacagatatt tgagcatgtg ttcctgggct 1080
cagaatggaa tgcctccaac ttagaggact tacagaaccg aggggtacgg tatatcttga 1140
atgtcactcg agagatagat aacttcttcc caggagtctt tgagtatcat aacattcggg 1200
tatatgatga agaggcaacg gatctcctgg cgtactggaa tgacacttac aaattcatct 1260
ctaaagcaaa gaaacatgga tctaaatgcc ttgtgcactg caaaatgggg gtgagtcgct 1320
cagcctccac cgtgattgcc tatgcaatga aggaatatgg ctggaatctg gaccgagcct 1380
atgactatgt gaaagaaaga cgaacggtaa ccaagcccaa cccaagcttc atgagacaac 1440
tggaagagta tcaggggatc ttgctggcaa gcttcctagg cttgattcat ggagggaggg 1500
acaagccctg ggagagagaa agcacagaat ttgagtcagt agatctggtt tccattcctg 1560
gttcacccctc ttgctgcaac cctgagaagt tacttcacat ttctcatcct tacctgaccc 1620
catctataaa a**tga**aaatca agagatccat ctcacagggt tattgtgaat aaaaatgtgt 1680
ttgaatgttt ataaaaaaaa aaaaaaaaaa a 1711

Fig. 29A

DSP-13 Polypeptide Sequence, 509 Amino Acids

Met Thr Leu Ser Thr Leu Ala Arg Lys Arg Lys Ala Pro Leu Ala Cys
Thr Cys Ser Leu Gly Gly Pro Asp Met Ile Pro Tyr Phe Ser Ala Asn
Ala Val Ile Ser Gln Asn Ala Ile Asn Gln Leu Ile Ser Glu Ser Phe
Leu Thr Val Lys Gly Ala Ala Leu Phe Leu Pro Arg Gly Asn Gly Ser
Ser Thr Pro Arg Ile Ser His Arg Arg Asn Lys His Ala Gly Asp Leu
Gln Gln His Leu Gln Ala Met Phe Ile Leu Leu Arg Pro Glu Asp Asn
Ile Arg Leu Ala Val Arg Leu Glu Ser Thr Tyr Gln Asn Arg Thr Arg
Tyr Met Val Val Val Ser Thr Asn Gly Arg Gln Asp Thr Glu Glu Ser
Ile Val Leu Gly Met Asp Phe Ser Ser Asn Asp Ser Ser Thr Cys Thr
Met Gly Leu Val Leu Pro Leu Trp Ser Asp Thr Leu Ile His Leu Asp
Gly Asp Gly Gly Phe Ser Val Ser Thr Asp Asn Arg Val His Ile Phe
Lys Pro Val Ser Val Gln Ala Met Trp Ser Ala Leu Gln Ser Leu His
Lys Ala Cys Glu Val Ala Arg Ala His Asn Tyr Tyr Pro Gly Ser Leu
Phe Leu Thr Trp Val Ser Tyr Tyr Glu Ser His Ile Asn Ser Asp Gln
Ser Ser Val Asn Glu Trp Asn Ala Met Gln Asp Val Gln Ser His Arg
Pro Asp Ser Pro Ala Leu Phe Thr Asp Ile Pro Thr Glu Arg Glu Arg
Thr Glu Arg Leu Ile Lys Thr Lys Leu Arg Glu Ile Met Met Gln Lys
Asp Leu Glu Asn Ile Thr Ser Lys Glu Ile Arg Thr Glu Leu Glu Met
Gln Met Val Cys Asn Leu Arg Glu Phe Lys Glu Phe Ile Asp Asn Glu
Met Ile Val Ile Leu Gly Gln Met Asp Ser Pro Thr Gln Ile Phe Glu
His Val Phe Leu Gly Ser Glu Trp Asn Ala Ser Asn Leu Glu Asp Leu
Gln Asn Arg Gly Val Arg Tyr Ile Leu Asn Val Thr Arg Glu Ile Asp
Asn Phe Phe Pro Gly Val Phe Glu Tyr His Asn Ile Arg Val Tyr Asp
Glu Glu Ala Thr Asp Leu Leu Ala Tyr Trp Asn Asp Thr Tyr Lys Phe
Ile Ser Lys Ala Lys Lys His Gly Ser Lys Cys Leu Val His Cys Lys
Met Gly Val Ser Arg Ser Ala Ser Thr Val Ile Ala Tyr Ala Met Lys
Glu Tyr Gly Trp Asn Leu Asp Arg Ala Tyr Asp Tyr Val Lys Glu Arg
Arg Thr Val Thr Lys Pro Asn Pro Ser Phe Met Arg Gln Leu Glu Glu
Tyr Gln Gly Ile Leu Leu Ala Ser Phe Leu Gly Leu Ile His Gly Gly
Arg Asp Lys Pro Trp Gly Glu Lys Ser Thr Glu Phe Glu Ser Val Asp
Leu Val Ser Ile Pro Gly Ser Pro Ser Cys Cys Asn Pro Glu Lys Leu
Leu His Ile Ser His Pro Tyr Leu Thr Pro Ser Ile Lys

Fig. 29B

DSP-14 Encoding Polynucleotide

ggccagtggg ggtggctggg cgtgcggctg ctacatgccc cacggaccag aacctcccga 60
cgcggccagg ccccggcaca cccagctgca gaaaggagag aaaatccctt ggctctaaaa 120
tgacatctgg agaagtgaag acaagcctca agaatgccta ctcatctgcc aagaggctgt 180
cgccgaagat ggaggaggaa ggggaggagg aggactactg cacccctgga gcctttgagc 240
tggagcggct cttctggaag ggcagtcccc agtacaccca cgtcaacgag gtctggccca 300
agctctacat tggcgatgag gcgacggcgc tggaccgcta taggctgcag aaggcggggt 360
tcacgcacgt gctgaacgcg gcccacggcc gctggaacgt ggacactggg cccgactact 420
accgcgacat ggacatccag taccacggcg tggaggccga cgacctgccc accttcgacc 480
tcagtgtctt cttctacccg gcggcagcct tcatcgacag agcgctaagc gacgaccaca 540
gtaagatcct ggttcactgc gtcatgggcc gcagccggtc agccaccctg gtcctggcct 600
acctgatgat ccacaaggac atgaccctgg tggacgccat ccagcaagtg gccaagaacc 660
gctgcgtcct cccgaaccgg ggctttttga agcagctccg ggagctggac aagcagctgg 720
tgcagcagag gcgacggtcc cagcgccagg acggtgagga ggaggatggc agggagctgt 780
aggcccgact cacagggcca gcagaggcac ttggggacag aggggagagg cagaacatag 840
ccctggccta ggactccaga gaagggatgg tgaaaccgaa gctcgactct tccaaaccat 900
cttgttcaac ttccccatgt gtgctgggga cagggaggac ccagagctgc ccccgggcag 960
agctgagcgc tcagcctctc agcaaaatgg gagggacggg ctcccccggct ctgggtcaca 1020
gaggagcatg ccacgctgca ccaagtctcc tgctttggtt ttgttttttt ggtgagaagg 1080
aagagggaaa aagattttta aaatgtgtag gcagtatgtt gtgattaaac gtttggcttt 1140
gtccaaaaaa aaaaaaaaaa aaaaa 1165

Fig. 30A

DSP-14 Polypeptide Sequence

Met Thr Ser Gly Glu Val Lys Thr Ser Leu Lys Asn Ala Tyr Ser Ser
Ala Lys Arg Leu Ser Pro Lys Met Glu Glu Glu Gly Glu Glu Glu Asp
Tyr Cys Thr Pro Gly Ala Phe Glu Leu Glu Arg Leu Phe Trp Lys Gly
Ser Pro Gln Tyr Thr His Val Asn Glu Val Trp Pro Lys Leu Tyr Ile
Gly Asp Glu Ala Thr Ala Leu Asp Arg Tyr Arg Leu Gln Lys Ala Gly
Phe Thr His Val Leu Asn Ala Ala His Gly Arg Trp Asn Val Asp Thr
Gly Pro Asp Tyr Tyr Arg Asp Met Asp Ile Gln Tyr His Gly Val Glu
Ala Asp Asp Leu Pro Thr Phe Asp Leu Ser Val Phe Phe Tyr Pro Ala
Ala Ala Phe Ile Asp Arg Ala Leu Ser Asp Asp His Ser Lys Ile Leu
Val His Cys Val Met Gly Arg Ser Arg Ser Ala Thr Leu Val Leu Ala
Tyr Leu Met Ile His Lys Asp Met Thr Leu Val Asp Ala Ile Gln Gln
Val Ala Lys Asn Arg Cys Val Leu Pro Asn Arg Gly Phe Leu Lys Gln
Leu Arg Glu Leu Asp Lys Gln Leu Val Gln Gln Arg Arg Arg Ser Gln
Arg Gln Asp Gly Glu Glu Glu Asp Gly Arg Glu Leu

Fig. 30B

MODULATION OF BIOLOGICAL SIGNAL TRANSDUCTION BY RNA INTERFERENCE

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 60/383,249 filed May 23, 2002, and U.S. Provisional Patent Application No. 60/462,942 filed Apr. 14, 2003, which are incorporated herein by reference in their entirety.

BACKGROUND OF THE INVENTION

[0002] 1. Technical Field

[0003] The present invention relates generally to compositions and methods useful for treating conditions associated with defects in cell proliferation, cell differentiation, and cell survival. The invention is more particularly related to double-stranded RNA polynucleotides that interfere with expression of protein tyrosine phosphatases, and polypeptide variants thereof. The invention is also particularly related to double-stranded RNA polynucleotides that interfere with expression of MAP kinases and MAP kinase kinases and chemotherapeutic target polypeptides, and polypeptide variants thereof. The present invention is also related to the use of such RNA polynucleotides to alter activation of signal transduction pathway components or to alter cellular metabolic processes that lead to proliferative responses, cell differentiation and development, and cell survival.

[0004] 2. Description of the Related Art

[0005] Reversible protein tyrosine phosphorylation, coordinated by the action of protein tyrosine kinases (PTKs) that phosphorylate certain tyrosine residues in polypeptides, and protein tyrosine phosphatases (PTPs) that dephosphorylate certain phosphotyrosine residues, is a key mechanism in regulating many cellular activities. It is becoming apparent that the diversity and complexity of the PTPs and PTKs are comparable, and that PTPs are equally important in delivering both positive and negative signals for proper function of cellular machinery. Regulated tyrosine phosphorylation contributes to specific pathways for biological signal transduction, including those associated with cell division, cell survival, apoptosis, proliferation and differentiation. Defects and/or malfunctions in these pathways may underlie certain disease conditions for which effective means for intervention remain elusive, including for example, malignancy, autoimmune disorders, diabetes, obesity and infection.

[0006] The protein tyrosine phosphatase (PTP) family of enzymes consists of more than 100 structurally diverse proteins in vertebrates, including almost 40 human PTPs that have in common the conserved 250 amino acid PTP catalytic domain, but which display considerable variation in their non-catalytic segments (Charbonneau and Tonks, 1992 *Annu. Rev. Cell Biol.* 8:463-493; Tonks, 1993 *Semin. Cell Biol.* 4:373-453; Andersen et al., *Mol. Cell Biol.* 21:7117-36 (2001)). This structural diversity presumably reflects the diversity of physiological roles of individual PTP family members, which in certain cases have been demonstrated to have specific functions in growth, development and differentiation (Desai et al., 1996 *Cell* 84:599-609; Kishihara et al., 1993 *Cell* 74:143-156; Perkins et al., 1992 *Cell* 70:225-236; Pingel and Thomas, 1989 *Cell* 58:1055-1065; Schultz et al., 1993 *Cell* 73:1445-1454). The PTP family includes receptor-like and non-transmembrane enzymes that exhibit exquisite substrate specificity in vivo and that are involved in regulating a wide variety of cellular signaling pathways (Andersen et al., *Mol. Cell. Biol.* 21:7117 (2001); Tonks and Neel, *Curr. Opin. Cell Biol.* 13:182 (2001)). PTPs thus participate in a variety of physiologic functions, providing a number of opportunities for therapeutic intervention in physiologic processes through alteration (i.e., a statistically significant increase or decrease) or modulation (e.g., up-regulation or down-regulation) of PTP activity.

[0007] Although recent studies have also generated considerable information regarding the structure, expression and regulation of PTPs, the nature of many tyrosine phosphorylated substrates through which the PTPs exert their effects remains to be determined. Studies with a limited number of synthetic phosphopeptide substrates have demonstrated some differences in the substrate selectivities of different PTPs (Cho et al., 1993 *Protein Sci.* 2: 977-984; Dechert et al., 1995 *Eur. J. Biochem.* 231:673-681). Analyses of PTP-mediated dephosphorylation of PTP substrates suggest that catalytic activity may be favored by the presence of certain amino acid residues at specific positions in the substrate polypeptide relative to the phosphorylated tyrosine residue (Salmeen et al., 2000 *Molecular Cell* 6:1401; Myers et al., 2001 *J. Biol. Chem.* 276:47771; Myers et al., 1997 *Proc. Natl. Acad. Sci. USA* 94:9052; Ruzzene et al., 1993 *Eur. J. Biochem.* 211:289295; Zhang et al., 1994 *Biochemistry* 33:2285-2290). Thus, although the physiological relevance of the substrates used in these studies is unclear, PTPs display a certain level of substrate selectivity in vitro.

[0008] The PTP family of enzymes contains a common evolutionarily conserved segment of approximately 250 amino acids known as the PTP catalytic domain. Within this conserved domain is a unique signature sequence motif, CX_5R (SEQ ID NO:______), that is invariant among all PTPs. In a majority of PTPs, an 11 amino acid conserved sequence ([IIV]HCXAGXXR[S/T)G (SEQ ID NO:______)) containing the signature sequence motif is found. The cysteine residue in this motif is invariant in members of the family and is essential for catalysis of the phosphotyrosine dephosphorylation reaction. It functions as a nucleophile to attack the phosphate moiety present on a phosphotyrosine residue of the incoming substrate. If the cysteine residue is altered by site-directed mutagenesis to serine (e.g., in cysteine-to-serine or "CS" mutants) or alanine (e.g., cysteine-to-alanine or "CA" mutants), the resulting PTP is catalytically deficient but retains the ability to complex with, or bind, its substrate, at least in vitro.

[0009] The CS mutant of one PTP, PTP1B (PTP-1B), is an example of such a PTP. Catalytically deficient mutants of such enzymes that are capable of forming stable complexes with phosphotyrosyl polypeptide substrates may be derived by mutating a wildtype protein tyrosine phosphatase catalytic domain invariant aspartate residue and replacing it with an amino acid that does not cause significant alteration of the Km of the enzyme but that results in a reduction in Kcat, as disclosed, for example, in U.S. Pat. Nos. 5,912,138 and 5,951,979, in U.S. application Ser. No. 09/323,426 and in PCT/US97/13016 and PCT/JUS00/14211. For instance,

mutation of Asp 181 in PTP1B to alanine to create the aspartate-to-alanine (D to A or DA) mutant PTP1B-D181A results in a PTP1B "substrate trapping" mutant enzyme that forms a stable complex with its phosphotyrosyl polypeptide substrate (e.g., Flint et al., 1997 *Proc. Natl. Acad. Sci.* 94:1680). Substrates of other PTPs can be identified using a similar substrate trapping approach, for example substrates of the PTP family members PTP-PEST (Garton et al., 1996 *J. Mol. Cell. Biol.* 16:6408), TCPTP (Tiganis et al., 1998 *Mol. Cell Biol.* 18:1622), PTP-HSCF (Spencer et al., 1997 *J. Cell Biol.* 138:845), and PTP-H1 (Zhang et al., 1999 *J. Biol. Chem.* 274:17806).

[0010] Mitogen-activated protein kinases (MAP-kinases) are components of conserved cellular signal transduction pathways that have a variety of conserved members and that that are integral to the cell's response to stimuli such as growth factors, hormones, cytokines, and environmental stresses. MAP-kinases are activated by phosphorylation by MAP-kinase kinases at a dual phosphorylation motif that has the sequence Thr-X-Tyr, in which phosphorylation at the tyrosine and threonine residues is required for activity. Activated MAP-kinases phosphorylate several transduction targets, including effector protein kinases and transcription factors. Inactivation of MAP-kinases is mediated by dephosphorylation at the Thr-X-Tyr site by dual-specificity phosphatases referred to as MAP-kinase phosphatases. In higher eukaryotes, the physiological role of MAP-kinase signaling has been correlated with cellular events such as proliferation, oncogenesis, development, and differentiation. Accordingly, the ability to regulate signal transduction via these pathways could lead to the development of treatments and preventive therapies for human diseases associated with MAP-kinase signaling, such as cancer.

[0011] Dual-specificity protein tyrosine phosphatases (dual-specificity phosphatases) dephosphorylate both phosphotyrosine and phosphothreonine/serine residues (Walton et al., *Ann. Rev. Biochem.* 62:101-120, 1993). More than 50 dual-specificity phosphatases that dephosphorylate and inactivate a MAP-kinase have been identified (Shen et al., *Proc. Natl. Acad. Sci. USA* 98:13613-18 (2001)), including MKP-1 (WO 97/00315; Keyse and Emslie, *Nature* 59:644-647 (1992)); MKP-2 (WO97/00315); MKP-4, MKP-5, MKP-7, Hb5 (WO 97/06245); PAC1 (Ward et al., *Nature* 367:651-654 (1994)); HVH2 (Guan and Butch, *J. Biol. Chem.* 270:7197-7203 (1995)); and PYST1 (Groom et al., *EMBO J.* 15:3621-3632 (1996)). These dual-specificity phosphatases differ in expression, tissue and subcellular distribution, and specificity for MAP-kinase family members. Expression of certain dual-specificity phosphatases is induced by stress or mitogens, but others appear to be expressed constitutively in specific cell types. The regulation of dual-specificity phosphatase expression and activity is critical for control of MAP-kinase mediated cellular functions, including cell proliferation, cell differentiation and cell survival. For example, dual-specificity phosphatases may function as negative regulators of cell proliferation. It is likely that there are many such dual-specificity phosphatases, with varying specificity with regard to cell type or activation.

[0012] In contrast to the role of most dual-specificity phosphatases to inactivate MAP-kinases, one enzyme, herein referred to as dual-specificity phosphatase 3 (DSP-3), has been reported to have the capability to function as a selective activator of the JNK MAP-kinase signaling pathway (Shen et al., supra; WO 01/21812). DSP-3 appears also to affect the activity of other kinases involved in the JNK pathway (Shen et al., supra; WO 01/21812). For example, overexpression of DSP-3 leads to activation of MKK4, a MAP-kinase kinase that functions upstream of JNK (Shen et al., supra; Lawler et al., *Curr. Biol.* 8:1387-90 (1998); Yang et al., *Proc. Natl. Acad. Sci. USA* 94: 3004-3009 (1997)).

[0013] Activation of JNK is believed to be involved in several physiological processes, including embryonic morphogenesis, cell survival, and apoptosis. A number of JNK signaling pathway substrates have been identified, including c-Jun, ATF2, ELK-1 and others. JNK signaling has also been associated with various disease conditions, such as tumor development, ischemia and reperfusion injury, diabetes, hyperglycemia-induced apoptosis, cardiac hypertrophy, inflammation, and neurodegenerative disorders.

[0014] One non-transmembrane PTP, PTP1B, recognizes several tyrosine-phosphorylated proteins as substrates, many of which are involved in human disease. For example, therapeutic inhibition of PTP1B in the insulin signaling pathway may serve to augment insulin action, thereby ameliorating the state of insulin resistance common in Type II diabetes patients. PTP1B acts as a negative regulator of signaling that is initiated by several growth factor/hormone receptor PTKs, including p210 Bcr-Abl (LaMontagne et al., *Mol. Cell Biol.* 18:2965-75 (1998); LaMontagne et al., *Proc. Natl. Acad. Sci. USA* 95:14094-99 (1998)), receptor tyrosine kinases, such as EGF receptor, PDGF receptor, and insulin receptor (IR) (Tonks et al., *Curr. Opin. Cell Biol.* 13:182-95 (2001)), and JAK family members such as Jak2 and others (Myers et al., *J. Biol. Chem.* 276:47771-74 (2001)), as well as signaling events induced by cytokines (Tonks and Neel, 2001). Activity of PTP1B is regulated by modifications of several amino acid residues, such as phosphorylation of Ser residues (Brautigan and Pinault, 1993; Dadke et al., 2001; Flint et al., 1993), and oxidation of the active Cys residue in its catalytic motif (Lee et al., 1998; Meng et al., 2002) which is evolutionary conserved among protein tyrosine phosphatases and dual phosphatase family members (Andersen et al., 2001).

[0015] Disruption of the murine PTP1B gene homolog in a knock-out mouse model results in $PTP1B^{-/-}$ mice exhibiting enhanced insulin sensitivity, decreased levels of circulating insulin and glucose, and resistance to weight gain even on a high-fat diet, relative to control animals having at least one functional PTP1B gene (Elchebly et al., *Science* 283:1544 (1999)). Insulin receptor hyperphosphorylation has also been detected in certain tissues of PTP1B deficient mice, consistent with a PTP1B contribution to the physiologic regulation of insulin and glucose metabolism (Id.). PTP-1B-deficient mice exhibit decreased adiposity (reduced fat cell mass but not fat cell number), increased basal metabolic rate and energy expenditure, and enhanced insulin-stimulated glucose utilization (Klaman et al., 2000 *Mol. Cell. Biol.* 20:5479). Additionally, altered PTP activity has been correlated with impaired glucose metabolism in other biological systems (e.g., McGuire et al., *Diabetes* 40:939 (1991); Myerovitch et al., *J. Clin. Invest.* 84:976 (1989); Sredy et al., *Metabolism* 44:1074 (1995)), including PTP involvement in biological signal transduction via the insulin receptor (see, e.g., WO 99/46268 and references cited therein).

[0016] An integration of crystallographic, kinetic, and PTP1B-peptide binding assays illustrated the interaction of PTP1B and insulin receptor (IR) (Salmeen et al., *Mol. Cell* 6:1401-12 (2000)). The insulin receptor (IR) comprises two extracellular α subunits and two transmembrane β subunits. Activation of the receptor results in autophosphorylation of tyrosine residues in both β subunits, each of which contains a protein kinase domain. Extensive interactions that form between PTP1B and insulin receptor kinase (IRK) encompass tandem pTyr residues at 1162 and 1163 of IRK, such that pTyr-1162 is located in the active site of PTP1B (id.). The Asp/Glu-pTyr-pTyr-Arg/Lys motif has been implicated for optimal recognition by PTP1B for IRK. This motif is also present in other receptor PTKs, including Trk, FGFR, and Axl. In addition, this motif is found in the JAK family of PTKs, members of which transmit signals from cytokine receptors, including a classic cytokine receptor that is recognized by the satiety hormone leptin (Touw et al., *Mol. Cell. Endocrinol.* 160:1-9 (2000)).

[0017] Changes in the expression levels of PTP1B have been observed in several human diseases, particularly in diseases associated with disruption of the normal patterns of tyrosine phosphorylation. For example, the expression of PTP1B is induced specifically by the p210 Bcr-Abl oncoprotein, a PTK that is directly responsible for the initial manifestations of chronic myelogenous leukemia (CML) (LaMontagne et al., *Mol. Cell. Biol.* 18:2965-75 (1998); LaMontagne et al., *Proc. Natl. Acad. Sci. USA* 95:14094-99 (1998)). Expression of PTPB1 in response to this oncoprotein is regulated, in part, by transcription factors Sp1, Sp3, and Egr-1 (Fukada et al., *J. Biol. Chem.* 276:25512-19 (2001)). These transcription factors have been shown to bind to a p210 Bcr-Abl responsive sequence (PRS) in the human PTP1B promoter, located between 49 to −37 base pairs from the transcription start site, but do not appear to mediate certain additional, independent PTP1B transcriptional events, for which neither transcription factor(s) nor transcription factor recognition element(s) have been defined (id.).

[0018] Diabetes mellitus is a common, degenerative disease affecting 5-10% of the human population in developed countries, and in many countries, it may be one of the five leading causes of death. Approximately 2% of the world's population has diabetes, the overwhelming majority of cases (>97%) being type 2 diabetes and the remainder being type 1. In type 1 diabetes, which is frequently diagnosed in children or young adults, insulin production by pancreatic islet beta cells is destroyed. Type 2 diabetes, or "late onset" or "adult onset" diabetes, is a complex metabolic disorder in which cells and tissues cannot effectively use available insulin; in some cases insulin production is also inadequate. At the cellular level, the degenerative phenotype that may be characteristic of late onset diabetes mellitus includes, for example, impaired insulin secretion and decreased insulin sensitivity, i.e., an impaired response to insulin.

[0019] Studies have shown that diabetes mellitus may be preceded by or is associated with certain related disorders. For example, an estimated forty million individuals in the U.S. suffer from late onset impaired glucose tolerance (IGT). IGT patients fail to respond to glucose with increased insulin secretion. Each year a small percentage (5-10%) of IGT individuals progress to insulin deficient non-insulin dependent diabetes (NIDDM). Some of these individuals further progress to insulin dependent diabetes mellitus (IDDM). NIDDM and IDDM are associated with decreased release of insulin by pancreatic beta cells and/or a decreased response to insulin by cells and tissues that normally exhibit insulin sensitivity. Other symptoms of diabetes mellitus and conditions that precede or are associated with diabetes mellitus include obesity, vascular pathologies, and various neuropathies, including blindness and deafness.

[0020] Type 1 diabetes is treated with lifelong insulin therapy, which is often associated with undesirable side effects such as weight gain and an increased risk of hypoglycemia. Current therapies for type 2 diabetes (NIDDM) include altered diet, exercise therapy, and pharmacological intervention with injected insulin or oral agents that are designed to lower blood glucose levels. Examples of such presently available oral agents include sulfonylureas, biguanides, thiazolidinediones, repaglinide, and acarbose, each of which alters insulin and/or glucose levels. None of the current pharmacological therapies, however, controls the disease over its full course, nor do any of the current therapies correct all of the physiological abnormalities in type 2 NIDDM, such as impaired insulin secretion, insulin resistance, and excessive hepatic glucose output. In addition, treatment failures are common with these agents, such that multi-drug therapy is frequently necessary.

[0021] In certain metabolic diseases or disorders, one or more biochemical processes, which may be either anabolic or catabolic (e.g., build-up or breakdown of substances, respectively), are altered (e.g., increased or decreased in a statistically significant manner) or modulated (e.g., up- or down-regulated to a statistically significant degree) relative to the levels at which they occur in a disease-free or normal subject such as an appropriate control individual. The alteration may result from an increase or decrease in a substrate, enzyme, cofactor, or any other component in any biochemical reaction involved in a particular process. Altered (i.e., increased or decreased in a statistically significant manner relative to a normal state) PTP activity can underlie certain disorders and suggests a PTP role in certain metabolic diseases.

[0022] RNA interference (RNAi) is a polynucleotide sequence-specific, post-transcriptional gene silencing mechanism effected by double-stranded RNA that results in degradation of a specific messenger RNA (mRNA), thereby reducing the expression of a desired target polypeptide encoded by the mRNA (see, e.g., WO 99/32619; WO 01/75164; U.S. Pat. No. 6,506,559; Fire et al., *Nature* 391:806-11 (1998); Sharp, *Genes Dev.* 13:139-41 (1999); Elbashir et al. *Nature* 411:494-98 (2001); Harborth et al., *J. Cell Sci.* 114:4557-65 (2001)). RNAi is mediated by double-stranded polynucleotides as also described hereinbelow, for example, double-stranded RNA (dsRNA), having sequences that correspond to exonic sequences encoding portions of the polypeptides for which expression is compromised. RNAi reportedly is not effected by double-stranded RNA polynucleotides that share sequence identity with intronic or promoter sequences (Elbashir et al., 2001). RNAi pathways have been best characterized in Drosophila and *Caenorhabditis elegans*, but "small interfering RNA" (siRNA) polynucleotides that interfere with expression of specific polypeptides in higher eukaryotes such as mammals (including humans) have also been considered (e.g., Tuschl, 2001 *Chembiochem.* 2:239-245; Sharp, 2001 *Genes Dev.* 15:485;

Bernstein et al., 2001 *RNA* 7:1509; Zamore, 2002 *Science* 296:1265; Plasterk, 2002 *Science* 296:1263; Zamore 2001 *Nat. Struct. Biol.* 8:746; Matzke et al., 2001 *Science* 293:1080; Scadden et al., 2001 *EMBO Rep.* 2:1107).

[0023] According to a current non-limiting model, the RNAi pathway is initiated by ATP-dependent, processive cleavage of long dsRNA into double-stranded fragments of about 18-27 (e.g., 19, 20, 21, 22, 23, 24, 25, 26, etc.) nucleotide base pairs in length, called small interfering RNAs (siRNAs) (see review by Hutvagner et al., *Curr. Opin. Gen. Dev.* 12:225-32 (2002); Elbashir et al., 2001; Nykänen et al., *Cell* 107:309-21 (2001); Zamore et al., *Cell* 101:25-33 (2000); Bass, *Cell* 101:235-38 (2000)). In Drosophila, an enzyme known as "Dicer" cleaves the longer double-stranded RNA into siRNAs; Dicer belongs to the RNase III family of dsRNA-specific endonucleases (WO 01/68836; Bernstein et al., *Nature* 409:363-66 (2001)). Further according to this non-limiting model, the siRNA duplexes are incorporated into a protein complex, followed by ATP-dependent unwinding of the siRNA, which then generates an active RNA-induced silencing complex (RISC) (WO 01/68836). The complex recognizes and cleaves a target RNA that is complementary to the guide strand of the siRNA, thus interfering with expression of a specific protein (Hutvagner et al., supra).

[0024] In *C. elegans* and Drosophila, RNAi may be mediated by long double-stranded RNA polynucleotides (WO 99/32619; WO 01/75164; Fire et al., 1998; Clemens et al., *Proc. Natl. Acad. Sci. USA* 97:6499-6503 (2000); Kisielow et al., *Biochem. J.* 363:1-5 (2002); see also WO 01/92513 (RNAi-mediated silencing in yeast)). In mammalian cells, however, transfection with long dsRNA polynucleotides (i.e., greater than 30 base pairs) leads to activation of a non-specific sequence response that globally blocks the initiation of protein synthesis and causes mRNA degradation (Bass, *Nature* 411:428-29 (2001)). Transfection of human and other mammalian cells with double-stranded RNAs of about 18-27 nucleotide base pairs in length interferes in a sequence-specific manner with expression of particular polypeptides encoded by messenger RNAs (mRNA) containing corresponding nucleotide sequences (WO 01/75164; Elbashir et al., 2001; Elbashir et al., *Genes Dev.* 15:188-200 (2001)); Harborth et al., *J. Cell Sci.* 114:4557-65 (2001); Carthew et al., *Curr. Opin. Cell Biol.* 13:244-48 (2001); Mailand et al., *Nature Cell Biol.* Advance Online Publication (Mar. 18, 2002); Mailand et al. 2002 *Nature Cell Biol.* 4:317).

[0025] siRNA polynucleotides may offer certain advantages over other polynucleotides known to the art for use in sequence-specific alteration or modulation of gene expression to yield altered levels of an encoded polypeptide product. These advantages include lower effective siRNA polynucleotide concentrations, enhanced siRNA polynucleotide stability, and shorter siRNA polynucleotide oligonucleotide lengths relative to such other polynucleotides (e.g., antisense, ribozyme or triplex polynucleotides). By way of a brief background, "antisense" polynucleotides bind in a sequence-specific manner to target nucleic acids, such as mRNA or DNA, to prevent transcription of DNA or translation of the mRNA (see, e.g., U.S. Pat. No. 5,168,053; U.S. Pat. No. 5,190,931; U.S. Pat. No. 5,135,917; U.S. Pat. No. 5,087,617; see also, e.g., Clusel et al., 1993 *Nuc. Acids Res.* 21:3405-11, describing "dumbbell" antisense oligonucleotides). "Ribozyme" polynucleotides can be targeted to any RNA transcript and are capable of catalytically cleaving such transcripts, thus impairing translation of mRNA (see, e.g., U.S. Pat. No. 5,272,262; U.S. Pat. No. 5,144,019; and U.S. Pat. Nos. 5,168,053, 5,180,818, 5,116,742 and 5,093,246; U.S. 2002/193579). "Triplex" DNA molecules refers to single DNA strands that bind duplex DNA to form a colinear triplex molecule, thereby preventing transcription (see, e.g., U.S. Pat. No. 5,176,996, describing methods for making synthetic oligonucleotides that bind to target sites on duplex DNA). Such triple-stranded structures are unstable and form only transiently under physiological conditions. Because single-stranded polynucleotides do not readily diffuse into cells and are therefore susceptible to nuclease digestion, development of single-stranded DNA for antisense or triplex technologies often requires chemically modified nucleotides to improve stability and absorption by cells. siRNAs, by contrast, are readily taken up by intact cells, are effective at interfering with the expression of specific polypeptides at concentrations that are several orders of magnitude lower than those required for either antisense or ribozyme polynucleotides, and do not require the use of chemically modified nucleotides.

[0026] Importantly, despite a number of attempts to devise selection criteria for identifying oligonucleotide sequences that will be effective in siRNA based on features of the desired target mRNA sequence (e.g., percent GC content, position from the translation start codon, or sequence similarities based on an in silico sequence database search for homologues of the proposed siRNA) it is presently not possible to predict with any degree of confidence which of myriad possible candidate siRNA sequences that can be generated as nucleotide sequences that correspond to a desired target mRNA (e.g., dsRNA of about 18-27 nucleotide base pairs) will in fact exhibit siRNA activity (i.e., interference with expression of the polypeptide encoded by the mRNA). Instead, individual specific candidate siRNA polynucleotide or oligonucleotide sequences must be generated and tested to determine whether interference with expression of a desired polypeptide target can be effected. Accordingly, no routine method exists in the art for designing a siRNA polynucleotide that is, with certainty, capable of specifically altering the expression of a given PTP polypeptide, and thus for the overwhelming majority of PTPs no effective siRNA polynucleotide sequences are presently known.

[0027] Currently, therefore, desirable goals for therapeutic regulation of biological signal transduction include modulation of PTP (e.g., PTP-1B, DSP-3, SHP-2, KAP, PRL-3, cdc14 or cdc25 or other PTP)-mediated cellular events include, inter alia, inhibition or potentiation of interactions among PTP-binding molecules, substrates and binding partners, or of other agents that regulate PTP activities. Accordingly, a need exists in the art for an improved ability to intervene in the regulation of phosphotyrosine signaling, including regulating PTPs by altering PTP catalytic activity, PTP binding to PTP substrate molecules, and/or PTP-encoding gene expression. An increased ability to so regulate PTPs may facilitate the development of methods for modulating the activity of proteins involved in phosphotyrosine signaling pathways and for treating conditions associated with such pathways. The present invention fulfills these needs and further provides other related advantages.

SUMMARY OF THE INVENTION

[0028] Briefly stated, the present invention provides siRNA compositions and methods for modulating biological signal transduction. In one aspect the present invention provides isolated small interfering RNA (siRNA) polynucleotide, comprising at least one nucleotide sequence selected from the sequences set forth in SEQ ID NOS: 4-7, 100-103, 105-108, 120-123, 125-128, 130-133, 140-143, 145-148, 150-153, 440-443, 445-448, 455-458, 460-463, 465-468, 470-473, 475-478, 480-483, 485-488, or 490-493, and the complementary polynucleotide thereto. The small interfering RNA polynucleotide is capable of interfering with expression of a polypeptide, which polypeptide comprises an amino acid sequence as set forth in a sequence SEQ ID NO: 779, SEQ ID NO 789, SEQ ID NO 791, SEQ ID NO 797, SEQ ID NO 799, SEQ ID NO 801, SEQ ID NO 803, SEQ ID NO 805, SEQ ID NO 807, SEQ ID NO 809, SEQ ID NO 811, or SEQ ID NO 813.

[0029] In certain embodiments, the nucleotide sequence of the siRNA polynucleotide differs by one, two, three or four nucleotides at any of positions 1-19 of a sequence selected from the sequences set forth in SEQ ID NOS: 4-7, 100-103, 105-108, 120-123, 125-128, 130-133, 140-143, 145-148, 150-153, 440-443, 445-448, 455-458, 460-463, 465-468, 470-473, 475-478, 480-483, 485-488, or 490-493. In other embodiments, the nucleotide sequence of the siRNA polynucleotide differs by at least two, three or four nucleotides at any of positions 1-19 of a sequence selected from the sequences set forth in SEQ ID NOS: 4-7, 100-103, 105-108, 120-123, 125-128, 130-133, 140-143, 145-148, 150-153, 440-443, 445-448, 455-458, 460-463, 465-468, 470-473, 475-478, 480-483, 485-488, or 490-493. In particular embodiments the invention provides an isolated siRNA polynucleotide comprising a nucleotide sequence selected from SEQ ID NOS: 4, or the complement thereof; from SEQ ID NOS: 100, 105, or the complement thereof; from SEQ ID NOS: 120, 125, or 130; or the complement thereof, from SEQ ID NOS: 140, 145, or 150, or the complement thereof; from SEQ ID NOS: 440 or 445, or the complement thereof; from SEQ ID NOS: 455 or 460; from SEQ ID NO: 465, or the complement thereof; from SEQ ID NOS: 470 or 475, or the complement thereof; from SEQ ID NOS: 480, 485, or 490, or the complement thereof.

[0030] In certain embodiments the invention provides the above siRNA polynucleotides that comprise at least one synthetic nucleotide analogue of a naturally occurring nucleotide. In certain other embodiments, the siRNA polynucleotide is linked to a detectable label, wherein the detectable label is a reporter molecule. In particular embodiments, the reporter molecule is a dye, a radionuclide, a luminescent group, a fluorescent group, or biotin. In other particular embodiments, the fluorescent group is fluorescein isothiocyanate and in other particular embodiments, the detectable label is a magnetic particle.

[0031] The invention also provides a pharmaceutical composition comprising an siRNA polynucleotide selectted from the sequences set forth in SEQ ID NOS: 4-7, 100-103, 105-108, 120-123, 125-128, 130-133, 140-143, 145-148, 150-153, 440-443, 445-448, 455-458, 460-463, 465-468, 470-473, 475-478, 480-483, 485-488, or 490-493, and a physiologically acceptable carrier. In particular embodiments, the the carrier comprises a liposome.

[0032] The invention also provides a recombinant nucleic acid construct comprising a polynucleotide that is capable of directing transcription of a small interfering RNA (siRNA), the polynucleotide comprising: (i) a first promoter; (ii) a second promoter; and (iii) at least one DNA polynucleotide segment comprising at least one nucleotide sequence selected from SEQ ID NOS: 4-7, 100-103, 105-108, 120-123, 125-128, 130-133, 140-143, 145-148, 150-153, 440-443, 445-448, 455-458, 460-463, 465-468, 470-473, 475-478, 480-483, 485-488, or 490-493, or a complement thereto, wherein each DNA polynucleotide segment and its complement are operably linked to at least one of the first and second promoters, and wherein the promoters are oriented to direct transcription of the DNA polynucleotide segment and its reverse complement. In certain embodiments, the recombinant nucleic acid construct comprises at least one enhancer that is selected from a first enhancer operably linked to the first promoter and a second enhancer operably linked to the second promoter. In certain other embodiments, the recombinant nucleic acid construct comprises at least one transcriptional terminator that is selected from (i) a first transcriptional terminator that is positioned in the construct to terminate transcription directed by the first promoter and (ii) a second transcriptional terminator that is positioned in the construct to terminate transcription directed by the second promoter. The invention also provides that the siRNA transcribed from the recombinant nucleic acid construct is capable of interfering with expression of a polypeptide, wherein the polypeptide comprises an amino acid sequence as set forth in a sequence selected from SEQ ID NO: 779, SEQ ID NO 789, SEQ ID NO 791, SEQ ID NO 797, SEQ ID NO 799, SEQ ID NO 801, SEQ ID NO 803, SEQ ID NO 805, SEQ ID NO 807, SEQ ID NO 809, SEQ ID NO 811, or SEQ ID NO 813.

[0033] The present invention also provides a recombinant nucleic acid construct comprising a polynucleotide that is capable of directing transcription of a small interfering RNA (siRNA), the polynucleotide comprising at least one promoter and a DNA polynucleotide segment, wherein the DNA polynucleotide segment is operably linked to the promoter, and wherein the DNA polynucleotide segment comprises (i) at least one DNA polynucleotide that comprises at least one nucleotide sequence selected from SEQ ID NOS: 4-7, 100-103, 105-108, 120-123, 125-128, 130-133, 140-143, 145-148, 150-153, 440-443, 445-448, 455-458, 460-463, 465-468, 470-473, 475-478, 480-483, 485-488, or 490-493, or a complement thereto; (ii) a spacer sequence comprising at least 4 nucleotides operably linked to the DNA polynucleotide of (i); and (iii) the reverse complement of the DNA polynucleotide of (i) operably linked to the spacer sequence. In certain embodiments, the siRNA polynucleotide transcribed from the recombinant nucleic acid construct comprises an overhang of at least one and no more than four nucleotides, the overhang being located immediately 3' to (iii). In certain particular embodiments, the spacer sequence comprises at least 9 nucleotides. In certain other specific embodiments the spacer sequence comprises two uridine nucleotides that are contiguous with (iii). In one embodiment, the recombinant nucleic acid construct comprises at least one transcriptional terminator that is operably linked to the DNA polynucleotide segment. The invention also provides a host cell that is transformed or transfected with such a recombinant nucleic acid construct as disclosed herein.

[0034] In one embodiment, the invention provides a pharmaceutical composition comprising an siRNA polynucleotide and a physiologically acceptable carrier, wherein the siRNA polynucleotide is selected from (i) an RNA polynucleotide that comprises at least one nucleotide sequence selected from SEQ ID NOS: 4-7, 100-103, 105-108, 120-123, 125-128, 130-133, 140-143, 145-148, 150-153, 440-443, 445-448, 455-458, 460-463, 465-468, 470-473, 475-478, 480-483, 485-488, or 490-493; (ii) an RNA polynucleotide that comprises at least one nucleotide sequence selected from SEQ ID NOS: 4-7, 100-103, 105-108, 120-123, 125-128, 130-133, 140-143, 145-148, 150-153, 440-443, 445-448, 455-458, 460-463, 465-468, 470-473, 475-478, 480-483, 485-488, or 490-493, and the complementary polynucleotide thereto; (iii) an RNA polynucleotide according to (i) or (ii) wherein the nucleotide sequence of the siRNA polynucleotide differs by one, two or three nucleotides at any of positions 1-19 of a sequence selected from SEQ ID NOS: 4-7, 100-103, 105-108, 120-123, 125-128, 130-133, 140-143, 145-148, 150-153, 440-443, 445-448, 455-458, 460-463, 465-468, 470-473, 475-478, 480-483, 485-488, or 490-493, or (iv) an RNA polynucleotide according to (i) or (ii) wherein the nucleotide sequence of the siRNA polynucleotide differs by two, three or four nucleotides at any of positions 1-19 of a sequence selected from the sequences set forth in SEQ ID NOS: 4-7, 100-103, 105-108, 120-123, 125-128, 130-133, 140-143, 145-148, 150-153, 440-443, 445-448, 455-458, 460-463, 465-468, 470-473, 475-478, 480-483, 485-488, or 490-493. In certain particular embodiments, the physiologically acceptable carrier comprises a liposome.

[0035] The present invention also provides a method for interfering with expression of a polypeptide, or variant thereof, comprising contacting a subject that comprises at least one cell which is capable of expressing the polypeptide with a siRNA polynucleotide for a time and under conditions sufficient to interfere with expression of the polypeptide, wherein: (a) the polypeptide comprises an amino acid sequence as set forth in a sequence selected from SEQ ID NO: 779, SEQ ID NO 789, SEQ ID NO 791, SEQ ID NO 797, SEQ ID NO 799, SEQ ID NO 801, SEQ ID NO 803, SEQ ID NO 805, SEQ ID NO 807, SEQ ID NO 809, SEQ ID NO 811, or SEQ ID NO 813, (b) the siRNA polynucleotide is selected from (i) an RNA polynucleotide which comprises at least one nucleotide sequence selected from SEQ ID NOS: 4-7, 100-103, 105-108, 120-123, 125-128, 130-133, 140-143, 145-148, 150-153, 440-443, 445-448, 455-458, 460-463, 465-468, 470-473, 475-478, 480-483, 485-488, or 490-493, (ii) an RNA polynucleotide that comprises at least one nucleotide sequence selected from the group consisting of SEQ ID NOS: 4-7, 100-103, 105-108, 120-123, 125-128, 130-133, 140-143, 145-148, 150-153, 440-443, 445-448, 455-458, 460-463, 465-468, 470-473, 475-478, 480-483, 485-488, or 490-493, and the complementary polynucleotide thereto, (iii) an RNA polynucleotide according to (i) or (ii) wherein the nucleotide sequence of the siRNA polynucleotide differs by one, two or three nucleotides at any of positions 1-19 of a sequence selected from SEQ ID NOS: 4-7, 100-103, 105-108, 120-123, 125-128, 130-133, 140-143, 145-148, 150-153, 440-443, 445-448, 455-458, 460-463, 465-468, 470-473, 475-478, 480-483, 485-488, or 490-493, or (iv) an RNA polynucleotide according to (i) or (ii) wherein the nucleotide sequence of the siRNA polynucleotide differs by two, three or four nucleotides at any of positions 1-19 of a sequence selected from the group consisting of the sequences set forth in SEQ ID NOS: 4-7, 100-103, 105-108, 120-123, 125-128, 130-133, 140-143, 145-148, 150-153, 440-443, 445-448, 455-458, 460-463, 465-468, 470-473, 475-478, 480-483, 485-488, or 490-493.

[0036] In another embodiment, the invention provides a method for interfering with expression of a polypeptide that comprises an amino acid sequence as set forth in a sequence selected from SEQ ID NO: 779, SEQ ID NO 789, SEQ ID NO 791, SEQ ID NO 797, SEQ ID NO 799, SEQ ID NO 801, SEQ ID NO 803, SEQ ID NO 805, SEQ ID NO 807, SEQ ID NO 809, SEQ ID NO 811, or SEQ ID NO 813, or a variant of said polypeptide, said method comprising contacting, under conditions and for a time sufficient to interfere with expression of the polypeptide, (i) a subject that comprises at least one cell that is capable of expressing the polypeptide, and (ii) a recombinant nucleic acid construct according to the present invention as described herein.

[0037] In another embodiment, the invention provides a method for identifying a component of a signal transduction pathway comprising: (A) contacting a siRNA polynucleotide and a first biological sample comprising at least one cell that is capable of expressing a target polypeptide, or a variant of said polypeptide, under conditions and for a time sufficient for target polypeptide expression when the siRNA polynucleotide is not present, wherein (i) the target polypeptide comprises an amino acid sequence as set forth in a sequence selected from SEQ ID NO: 779, SEQ ID NO 789, SEQ ID NO 791, SEQ ID NO 797, SEQ ID NO 799, SEQ ID NO 801, SEQ ID NO 803, SEQ ID NO 805, SEQ ID NO 807, SEQ ID NO 809, SEQ ID NO 811, SEQ ID NO 813, SEQ ID NO 823, SEQ ID NO 825, or SEQ ID NO:827; (2) the siRNA polynucleotide is selected from (i) an RNA polynucleotide which comprises at least one nucleotide sequence selected from SEQ ID NOS: 4-7, 100-103, 105-108, 120-123, 125-128, 130-133, 140-143, 145-148, 150-153, 440-443, 445-448, 455-458, 460-463, 465-468, 470-473, 475-478, 480-483, 485-488, or 490-493, (ii) an RNA polynucleotide that comprises at least one nucleotide sequence selected from SEQ ID NOS: 4-7, 100-103, 105-108, 120-123, 125-128, 130-133, 140-143, 145-148, 150-153, 440-443, 445-448, 455-458, 460-463, 465-468, 470-473, 475-478, 480-483, 485-488, or 490-493, and the complementary polynucleotide thereto; (iii) an RNA polynucleotide according to (i) or (ii) wherein the nucleotide sequence of the siRNA polynucleotide differs by one, two or three nucleotides at any of positions 1-19 of a sequence selected from the group consisting of the sequences set forth in SEQ ID NOS: 4-7, 100-103, 105-108, 120-123, 125-128, 130-133, 140-143, 145-148, 150-153, 440-443, 445-448, 455-458, 460-463, 465-468, 470-473, 475-478, 480-483, 485-488, or 490-493, (iv) an RNA polynucleotide according to (i) or (ii) wherein the nucleotide sequence of the siRNA polynucleotide differs by two, three or four nucleotides at any of positions 1-19 of a sequence selected from the group consisting of the sequences set forth in SEQ ID NOS: 4-7, 100-103, 105-108, 120-123, 125-128, 130-133, 140-143, 145-148, 150-153, 440-443, 445-448, 455-458, 460-463, 465-468, 470-473, 475-478, 480-483, 485-488, or 490-493; and (B) comparing a level of phosphorylation of at least one protein that is capable of being phosphorylated in the cell with a level of phosphorylation of the protein in a control sample that has not been contacted with the siRNA poly-

nucleotide, wherein an altered level of phosphorylation of the protein in the presence of the siRNA polynucleotide relative to the level of phosphorylation of the protein in an absence of the siRNA polynucleotide indicates that the protein is a component of a signal transduction pathway. The invention also provides a small interfering RNA (siRNA) polynucleotide, comprising an RNA polynucleotide which comprises at least one nucleotide sequence selected from SEQ ID NOS:4-7, 100-103, 105-108, 120-123, 125-128, 130-133, 140-143, 145-148, 150-153, 440-443, 445-448, 455-458, 460-463, 465-468, 470-473, 475-478, 480-483, 485-488, or 490-493. Certain further embodiments relate to isolated siRNA polynucleotides that comprise nucleotide sequences having the above recited SEQ ID NOS, including compositions and methods for producing and therapeutically using such siRNA.

[0038] These and other embodiments of the present invention will become apparent upon reference to the following detailed description and attached drawings. All references disclosed herein are hereby incorporated by reference in their entireties as if each was incorporated individually. Also incorporated by reference are co-pending application Ser. No. ______ and Ser. No. ______ (attorney docket numbers 200125.441 and 200125.448, respectively), which have been filed concurrently.

BRIEF DESCRIPTION OF THE DRAWINGS

[0039] **FIG. 1** presents an immunoblot analysis of the expression of MKP-1 polypeptide in HeLa cells co-transfected with sequence-specific siRNA polynucleotides (MKPsi.1 (MKP.1, SEQ ID NO:______), lanes 1-3; MKPsi.2 (MKP.2, SEQ ID NO:______), lanes 4-6) and a non-specific sequence siRNA (CD45si.1, lanes 7-9). The immunoblot of HeLa cell extracts was probed with an anti-MKP-1 antibody (upper). A second SDS-PAGE gel in which the HeLa cell extracts were separated was stained with Coomassie Blue (lower).

[0040] **FIG. 2** shows an immunoblot analysis of 292-HEK cell lysates from cells co-transfected with FLAG®-DSP-11, FLAG®-DSP-18, FLAG®-DSP-3, and FLAG®-cdc14b expression vectors and siRNAs specific for DSP-11 or DSP-18. The presence of each polypeptide was detected using an anti-FLAG® antibody (Sigma-Aldrich, St. Louis, Mo.). The upper immunoblot shows the level of expression of FLAG®-DSP-11 in untransfected 293-HEK cells (lane 1); 293-HEK cells transfected with FLAG®-DSP-11 vector DNA only (buffer) (lane 2), siRNA DSP11.2 (lane 3), siRNA DSP11.4 (lane 4), siRNA DSP18.2 (lane 5), and siRNA DSP18.2 (lane 6); and the level of expression of 293-HEK cells transfected with FLAG®-DSP-18 vector DNA only (buffer) (lane 7); 293-HEK cells co-transfected with siRNA DSP11.2 (lane 8), siRNA DSP11.4 (lane 9), siRNA DSP18.2 (lane 10), and siRNA DSP18.2 (lane 11). The lower immunoblot shows the level of FLAG®-DSP-3 in untransfected 293-HEK cells (lane 1); 293-HEK cells transfected with FLAG®-DSP-3 vector DNA only (buffer) (lane 2); 293-HEK cells co-transfected with siRNA DSP11.2 (lane 3), siRNA DSP11.4 (lane 4), siRNA DSP18.2 (lane 5), and siRNA DSP18.2 (lane 6); and the level of expression of FLAG®-cdc14b in 293-HEK cells transfected with FLAG®-cdc14b vector DNA only (buffer) (lane 7); 293-HEK cells co-transfected with siRNA DSP11.2 (lane 8), siRNA DSP11.4 (lane 9), siRNA DSP18.2 (lane 10), and siRNA DSP18.2 (lane 11).

[0041] **FIG. 3** shows the effect on JNK activation by sequence-specific siRNA interference of DSP-3 polypeptide expression. HeLa cells were co-transfected with a DSP-3 recombinant expression vector and DSP3.1 siRNA (SEQ ID NO:1) or 60 pmoles (100 nM final) CD45.2 (SEQ ID NO:______). After transfection, cells were stimulated with either tumor necrosis factor-alpha (TNF-α) or epidermal growth factor (EGF) or were unstimulated (Unstim.).

[0042] **FIG. 4** shows the effect on JNK activation by sequence-specific siRNA interference of DSP-3 polypeptide expression. HeLa cells were co-transfected with a DSP-3 recombinant expression vector and DSP3.1 siRNA (SEQ ID NO:______) or 60 pmoles (100 nM final) CD45.2 (SEQ ID NO:______). After transfection, cells were stimulated with sorbitol.

[0043] **FIG. 5** presents an immunoblot analysis of ERK phosphorylation in HeLa cells co-transfected with a DSP-3 recombinant expression vector and DSP-3 specific siRNA DSP3.1, non-specific CD45.2 siRNA, or siRNA annealing buffer and then stimulated with TNF-α, EGF, sorbitol, and anisomycin. Lane 1: unstimulated cells transfected with DSP3.1 siRNA; lane 2: unstimulated cells transfected with CD45.2 siRNA; lane 3: cells transfected with DSP3.1 siRNA and stimulated with TNF-α; lane 4: cells transfected with CD45.2 siRNA and stimulated with TNF-α; lane 5: cells transfected with DSP3.1 siRNA and stimulated with EGF; lane 6: cells transfected with CD45.2 siRNA and stimulated with EGF; lane 7: unstimulated cells transfected with CD45.2 siRNA; lane 8: unstimulated cells transfected with siRNA annealing buffer; lane 9: cells transfected with DSP3.1 siRNA and stimulated with sorbitol; lane 10: cells transfected with CD45.2 siRNA and stimulated with sorbitol; lane 11; cells transfected with siRNA annealing buffer and stimulated with sorbitol; lane 12: cells transfected with DSP3.1 siRNA and stimulated with anisomycin; lane 13: cells transfected with CD45.2 siRNA and stimulated with anisomycin; lane 14: cells transfected with siRNA annealing buffer and stimulated with anisomycin.

[0044] **FIG. 6** shows an immunoblot analysis of FLAG®-tagged cdc14a expression in 293-HEK cells co-transfected with cdc14a.2 (lane 3); cdc14a.3 (lane 4); cdc14a.4 (land 5); cdc14a.5 (lane 6); DSP3.1 (lane 7); DSP3.2 (lane 8); cdc14b.3 (lane 9); cdc14b.4 (lane 10); MKP.2 (lane 11); CD45.3 (lane 12); no siRNA (lane 2). Untransfected cells were prepared as a control (lane 1). Expression was detected using an anti-FLAG® antibody (Sigma-Aldrich).

[0045] **FIG. 7** presents an immunoblot of expression of FLAG®-tagged dual specificity phosphatases in 293-HEK cells that were co-transfected with cdc14a.3 siRNA (denoted by +). Lanes 2 and 3: expression of FLAG®-tagged cdc14a; lanes 4 and 5: expression of FLAG®-tagged DSP-3; lanes 6 and 7: expression of FLAG®-tagged cdc14b; lanes 8 and 9: FLAG®-tagged DSP-11. The immunoblot to the right is an over-exposure of the immunoblot on the left to detect low concentrations of expressed polypeptides.

[0046] **FIG. 8** shows an immunoblot analysis of FLAG®-tagged cdc14b expression in 293-HEK cells co-transfected with cdc14b.3 (lane 3); cdc14b.4 (lane 4); cdc14a.3 (land 5); cdc14a.5 (lane 6); DSP3.1 (lane 7); DSP3.2 (lane 8); MKP.2 (lane 9); CD45.3 (lane 10); no siRNA (lane 2). Untransfected cells were prepared as a control (lane 1). Expression was detected using an anti-FLAG® antibody (Sigma-Aldrich).

[0047] **FIG. 9** presents an immunoblot of expression of FLAG®-tagged dual specificity phosphatases in 293-HEK cells co-transfected with either cdc14a or cdc14b specific siRNAs. Expression of the phosphatases was detected with an anti-FLAG® antibody. 293-HEK cells were transfected as follows: no expression vector or siRNA (lane 1); FLAG®-tagged cdc14b only (lane 2); FLAG®-tagged cdc14b and cdc14b.3 siRNA (lane 3); FLAG®-tagged cdc14b and cdc14b.4 (lane 5); FLAG®-tagged DSP-3 only (lane 5); FLAG®-tagged DSP-3 and cdc14b.3 siRNA (lane 6); FLAG®-tagged DSP3 and cdc14b.4 siRNA (lane 7); FLAG®-tagged DSP-3 and cdc14a.5 siRNA (lane 8); FLAG®-tagged DSP-11 only (lane 9); FLAG®-tagged DSP-11 and cdc14b.3 siRNA (land 10); FLAG®-tagged DSP-11 and cdc14b.4 siRNA (lane 11); and FLAG®-tagged DSP-11 and cdc14a.5 siRNA.

[0048] **FIG. 10** depicts the expression of cdc14b polypeptide in HeLa cells co-transfected with cdc14b.4 siRNA detected by immunocytochemistry (top right, 10× magnification; bottom right, 40× magnification) and in the absence of a specific siRNA (top left, 10× magnification; bottom right, 40× magnification).

[0049] **FIG. 11** depicts an immunoblot of the effect on endogenous expression of murine PTP1B by siRNAs specific for the murine PTP1B or the human PTP1B polynucleotide sequences. Expression was detected using a murine anti-PTP1B monoclonal antibody. Data are presented for two different clones of C57B16 #3 murine cells. Both clones were transfected with mPTP1B1.1 siRNA (lanes 3 and 8); MPTP1B1.2 (lanes 4 and 9); mPTP1B1.3 (lanes 5 and 10). One clone, C57B16 #3 clone 3, was transfected with hPTP1B1.1 (lane 6). Lane 2: untransfected C57B16 #3, clone 3; lane 7: untransfected C57B16 #3, clone 10.

[0050] **FIG. 12** presents an extended consensus cDNA sequence encoding prototypical DSP-18 (DSP-18pr) (**FIG. 12A**) [SEQ ID NO:______] and the deduced DSP-18pr amino acid sequence (**FIG. 12B**) [SEQ ID NO:______]. In **FIG. 12A**, initiating methionine (ATG) and stop (TGA) codons and intron/exon splice junctions are depicted in bold type with the splice donor sequences in bold without underscore, and the splice acceptor sequences in bold with underscore. In **FIG. 12B**, initiating methionine and the phosphatase active site are depicted in bold type.

[0051] **FIG. 13** presents nucleotide and amino acid sequences for a DSP-18 isoform, DSP-18a. **FIG. 13A** presents a cDNA sequence for DSP-18a [SEQ ID NO:______], with the start (ATG) and stop (TGA) codons and intron/exon splice junctions indicated in bold; intron/exon splice junctions are depicted in bold type with the splice donor sequences in bold without underscore and the splice acceptor sequences in bold with underscore. **FIG. 13B** presents the amino acid sequence of the DSP-18a polypeptide [SEQ ID NO:______] encoded by SEQ ID NO:______, with the phosphatase active site depicted in bold type.

[0052] **FIG. 14** presents nucleotide and amino acid sequences for a DSP-18 isoform, DSP-18b. **FIG. 14A** presents a cDNA sequence for DSP-18b [SEQ ID NO:______], with the start (ATG) and stop (TGA) codons and intron/exon splice junctions indicated in bold; intron/exon splice junctions are depicted in bold type with the splice donor sequences in bold without underscore and the splice acceptor sequences in bold with underscore. **FIG. 14B** presents the amino acid sequence of the DSP-18b polypeptide [SEQ ID NO:______] encoded by SEQ ID NO:______, with the phosphatase active site depicted in bold type.

[0053] **FIG. 15** presents nucleotide sequences for DSP-18 isoforms, DSP-18c and DSP-18d. **FIG. 15A** presents a cDNA sequence for DSP-18c [SEQ ID NO:______ with the start (ATG) and stop (TGA) codons and intron/exon splice junctions indicated in bold. **FIG. 15B** presents a cDNA sequence for DSP-18d [SEQ ID NO:______], with the start (ATG) and stop (TGA) codons and intron/exon splice junctions indicated in bold. DSP-18c [SEQ ID NO:______] encoded by SEQ ID NO:______, and DSP-18d [SEQ ID NO:______] encoded by SEQ ID NO:______, both share the 181 amino acid sequence encoded by the open reading frame of DSP-18a (see **FIG. 15**).

[0054] **FIG. 16** presents nucleotide and amino acid sequences for DSP-18 isoforms, DSP-18e and DSP-18f. **FIG. 16A** presents a cDNA sequence for DSP-18e [SEQ ID NO:______], with the start (ATG) and stop (TGA) codons and intron/exon splice junctions indicated in bold. **FIG. 16** presents the amino acid sequence of DSP-18e polypeptide [SEQ ID NO:______] encoded by SEQ ID NO:______, with the phosphatase active site sequence in boldface type.

[0055] **FIG. 17A** presents nucleotide and amino acid sequences for DSP-18f. **FIG. 17A** presents a cDNA sequence for DSP-18f [SEQ ID NO:______], with the start (ATG) and stop (TGA) codons and intron/exon splice junctions indicated in bold. **FIG. 17B** presents the amino acid sequence of DSP-18f polypeptide [SEQ ID NO:______] encoded by SEQ ID NO:______, with the phosphatase active site sequence in boldface type.

[0056] **FIG. 18** represents an immunoblot of cleavage of poly(ADP-ribose) polymerase (PARP) in HeLa cells transfected with cell division cycle protein sequence specific siRNA polynucleotides (10 nM). The upper immunoblot was probed with an antibody that specifically binds to cleaved PARP, and the lower immunoblot was probed with an anti-PARP antibody. The siRNA polynucleotides transfected into the HeLa cells were as follows: lanes 1 and 2, no siRNA; lanes 3 and 4, cdc14a.5; lanes 5 and 6, cdc14b.4; lanes 7 and 8 Cdc25A.2; lanes 9 and 10, Cdc25B.4; and lanes 11 and 12, Cdc25C.1.

[0057] **FIG. 19** depicts an immunoblot analysis of the expression of human PTP-1 B co-transfected into 1BKO+ HIR murine fibroblasts with human PTP-1B siRNA hairpin vectors. Expression was detected with an anti-human PTP1B antibody (h1B) (lower portion of immunoblot). As a protein expression control, cell lysates were probed with an anti-human insulin receptor (IR) antibody (upper portion of immunoblot).

[0058] **FIG. 20** illustrates insulin-induced activation of PKB/Akt in HepG2 cells following ablation of TC45 by RNA interference. **FIG. 20A** represents an immunoblot of serum-deprived Rat-1 and HEPG2 cells that were exposed to varying concentrations of insulin (INS) as shown. The insulin receptor (IR) was immunoprecipitated from cell lysates with an anti-IR-β antibody followed by immunoblotting with an anti-phosphotyrosine antibody (pY) (top panel); an anti-pYpY$^{1162/1163}$-IR-β antibody (middle panel); and an anti-IR β antibody. **FIG. 20B** represents an immu-

noblot of HepG2 cell lysates prepared from cells that were untransfected (control) or transfected with TCPTP1 siRNA (SEQ ID NO:______) (+siRNA). The lysates were immunoblotted with an anti-phospho-PKB/Akt antibody (p-AKT) (first immunoblot); anti-PKB/Akt antibody (AKT) (second immunoblot); anti-TC45 (TC45) antibody (third immunoblot); and an anti-PTP1B antibody (PTP1B). **FIG. 20C** represents a densitometric analysis of the gel image to illustrate the ratio of phosphorylated PKB/Akt to total PKB/Akt.

[0059] **FIG. 21** provides an immunoblot indicating that tyrosine phosphorylated IR-β is a substrate of TC45. HepG2 cells overexpressing wild-type (WT) or substrate trapping mutant (DA) forms of PTP1B (1B) and TC45 were either not treated with insulin (–INS) or stimulated with insulin for 5 minutes (+INS), lysed, separated by SDS-PAGE, and immunoprecipitated with anti-PTP1B antibody (FG6) or anti-TC45 antibody (CF4). The immunoprecipitates were immunoblotted with an anti-IR-β antibody (top panel, **FIG. 21A**); anti-PTP1B antibody FG6 (middle panel, **FIG. 21A**); and anti-TCPTP antibody CF4 (bottom panel, **FIG. 21A**). **FIG. 21B** depicts immunoblots of HepG2 cells that were serum-starved and untransfected (control) or transfected with TC45 siRNA (100 nM) and then stimulated with 10 nM insulin (INS) for the indicated times. The insulin receptor was immunoprecipitated from cell lysates with an anti-IR-β antibody, which was then immunoblotted with the following antibodies: anti-phosphotyrosine (p-Tyr) (first immunoblot); anti-pY^{972}-IR-β (second immunoblot); anti-pYp$Y^{1162/1163}$-IR-β (third immunoblot); and anti-IR-β (fourth immunoblot). **FIG. 21C** presents densitometric analyses of the gel image to show the ratio of phosphorylated IR-β to total IR-β for total phosphotyrosine (top panel); phosphorylation of Tyr 972 (middle panel); and phosphorylation of the activation loop tyrosines 1162 and 1163 (lower panel).

[0060] **FIG. 22** presents the results of an ELISA in which the level of insulin receptor (IR) phosphorylated tyrosine was measured in 293-HEK HIR cells transfected with 0, 0.5, 3, or 10 nM hPTP1B1.3 (H1.3, SEQ ID NO:______) (**FIG. 22A**) or mPTP1B1.1b (M1.1, SEQ ID NO:______) (**FIG. 22B**) siRNAs. The level of expression of human PTP1B in the cells was compared by immunoblot (see tables to right of each figure).

[0061] **FIG. 23** depicts the results of an ELISA in which the level of insulin receptor (IR) phosphorylated tyrosine was measured in 293-HEK HIR cells transfected with 0, 0.5, 3, or 10 nM siRNAs. The siRNA polynucleotides transfected into the cells included hPTP1B1.2 (H1.2, SEQ ID NO:______); hPTP1B1.3 (H1.3, SEQ ID NO:______); mPTP1B1.1b (M1.1, SEQ ID NO:______); and rPTP1B1.2 (R1.2, SEQ ID NO:______). Seventy-two hours after transfection, cells were exposed to insulin for 7 minutes at the designated concentrations. Cell lysates were prepared and coated onto 96-well plates and probed with an anti-pY-IR-β antibody.

[0062] **FIG. 24** depicts the results of an ELISA in which the level of insulin receptor (IR) phosphorylated tyrosine was measured in 293-HEK HIR cells transfected with 0, 0.5, 3, or 10 nM hTCPTP1.4 siRNA (TC1.4, SEQ ID NO:______) (**FIG. 24A**) and mPTP1B1.1b siRNA (M1.1, SEQ ID NO:______) (**FIG. 24B**). Seventy-two hours after transfection, cells were exposed to insulin for 7 minutes at the designated concentrations. Cell lysates were prepared and coated onto 96-well plates and probed with an anti-pY-IR-β antibody.

[0063] **FIG. 25** represents ELISA data from three separate experiments that represent the level of insulin receptor phosphorylation in cells transfected with hPTP1B1.3 and stimulated with 50 nM insulin (Ins). Each data point represents the average optical density measured in duplicate wells.

[0064] **FIG. 26** illustrates an MTT assay comparing proliferation of HCT-116 cells transfected with siRNAs specific for DSP-3 (dsp3.1 (SEQ ID NO:______) and dsp3.4 (SEQ ID NO:______)); cdc14a (a.3 (SEQ ID NO:______) and a.5 (SEQ ID NO:______)); SHP-2 (shp2.1 (SEQ ID NO:______) and shp2.2 (SEQ ID NO:______); and DHFR (DHFR.1 (SEQ ID NO:______). As a control, HCT-116 cells were transfected with nonspecific siRNA (scr.2 (SEQ ID NO:______)). Each bar represents the average optical density for six wells.

[0065] **FIG. 27** illustrates an MTT assay comparing proliferation of T47D cells transfected with siRNAs specific for DSP-3 (dsp3.1 (SEQ ID NO:______) and dsp3.4 (SEQ ID NO:______)); cdc14a (Cdc14a.3 (SEQ ID NO:______) and Cdc14a.5 (SEQ ID NO:______); SHP-2 (shp2.1 (SEQ ID NO:______) and shp2.2 (SEQ ID NO:______); and DHFR (DHFR.1 (SEQ ID NO:______). As a control, T47D cells were transfected with nonspecific siRNA (scr.2 (SEQ ID NO:______).

[0066] **FIG. 28** represents an immunoblot of cleavage of PARP in HCT-116 cells (**FIG. 28A**) and T47D (**FIG. 28B**) transfected with buffer only (lane 1); (scrb1.2 (SEQ ID NO:______) (lane 2); DSP3.1 (SEQ ID NO:______) (lane 3); DSP3.4 (SEQ ID NO:______) (lane 4); and DHFR.1 (lane 5).

[0067] **FIG. 29** presents nucleotide and amino acid sequences for DSP-13. **FIG. 29A** presents a cDNA sequence for DSP-13 [SEQ ID NO:______], with the start (ATG) and stop (TGA) codons indicated in bold and underlined. **FIG. 29B** presents the amino acid sequence of the DSP-13 polypeptide [SEQ ID NO:______] encoded by SEQ ID NO:______.

[0068] **FIG. 30** presents nucleotide and amino acid sequences for DSP-14. **FIG. 30A** presents a cDNA sequence for DSP-14 [SEQ ID NO:______], with the start (ATG) and stop (TGA) codons indicated in bold and underlined. **FIG. 30B** presents the amino acid sequence of the DSP-14 polypeptide [SEQ ID NO:______] encoded by SEQ ID NO:______.

DETAILED DESCRIPTION OF THE INVENTION

[0069] The present invention is directed in part to the unexpected discovery of short RNA polynucleotide sequences that are capable of specifically modulating expression of a desired polypeptide, such as a DSP-3, SHP-2, KAP, PRL-3, cdc14 or cdc25 polypeptide, or a variant of any such polypeptide. Without wishing to be bound by theory, the RNA polynucleotides of the present invention specifically reduce expression of a desired target polypeptide through recruitment of small interfering RNA (siRNA) mechanisms. In particular, and as described in

greater detail herein, according to the present invention there are provided compositions and methods that relate to the surprising identification of certain specific RNAi oligonucleotide sequences of 19, 20, 21, 22, 23, 24, 25, 26 or 27 nucleotides that can be derived from corresponding polynucleotide sequences encoding the desired DSP-3, SHP-2, KAP, PRL-3, cdc14, cdc25, or other specified target polypeptide. These sequences cannot be predicted through any algorithm, sequence alignment routine, or other systematic paradigm, but must instead be obtained through generation and functional testing for RNAi activity of actual candidate oligonucleotides, such as those disclosed for the first time herein.

[0070] In preferred embodiments of the invention, the siRNA polynucleotide interferes with expression of a DSP-3, SHP-2, KAP, PRL-3, cdc14, cdc25, or other herein specified target polypeptide or a variant thereof, and comprises a RNA oligonucleotide or RNA polynucleotide uniquely corresponding in its nucleotide base sequence to the sequence of a portion of a target polynucleotide encoding the target polypeptide, for instance, a target mRNA sequence or an exonic sequence encoding such mRNA. Hence, according to non-limiting theory, the siRNA polynucleotides of the present invention direct sequence-specific degradation of mRNA encoding a desired DSP-3, SHP-2, KAP, PRL-3, cdc14 or cdc25 target polypeptide, the expression of which is consequently compromised. As also described herein, certain embodiments of the invention relate to siRNA polynucleotides that specifically interfere with expression of PTPs that are dual specificity phosphatases, including DSP-3, DSP-11, DSP-13, DSP-14, and DSP-18; certain other embodiments relate to RNAi interference with expression of the MAP kinase kinase (MKK) target polypeptide MKK4; certain other embodiments relate to RNAi interference with expression of target polypeptides that interact with chemotherapeutic agents, for example, the target polypeptides dihydrofolate reductase (DHFR), thymidylate synthetase, and topoisomerase I. The invention relates in preferred embodiments to siRNA polynucleotides that interfere with expression of specific polypeptides in mammals, which in certain particularly preferred embodiments are humans and in certain other particularly preferred embodiments are non-human mammals.

[0071] Exemplary sequences for the target polypeptides described herein include, for instance, DSP-3 (WO 00/60092; SEQ ID NO:24 encoded by SEQ ID NO:23); cdc14A (e.g., GenBank Accession Nos. AF122013, AF064102, AF064103; Li et al., 1997 *J. Biol. Chem.* 272:29403; U.S. Pat. No. 6,331,614; e.g., SEQ ID NO:34 encoded by SEQ ID NO:33) or cdc14B (e.g., GenBank Accession Nos. AF064104, AF064105, AF023158; Li et al., 1997 *J. Biol. Chem.* 272:29403; e.g., SEQ ID NO:36 encoded by SEQ ID NO:35); cdc25A ((e.g., GenBank Accession Nos. NM_001789, AF527417, NM_133571); cdc25B (e.g., GenBank Accession Nos. NM_133572, NM_023117, NM_021872; NM_021872; M81934); and cdc25C (e.g., GenBank Accession Nos. NM_001790, NM_022809); PTPϵ (e.g., Genbank Accession Nos. NM_006504 (SEQ ID NOS: ______-______) and NM_130435(SEQ ID NOS: ______-______)); KAP (e.g., Genbank Accession No. L27711; Hannon et al., *Proc. Natl. Acad. Sci. USA* 91:1731-35 (1994); Demetrick et al., *Cytogenet. Cell Genet.* 69:190-92 (1995)); PRL-3 (e.g., Zhao et al., *Genomics* 35:172-81 (1996); Genbank Accession Nos. (NM_003479 (SEQ ID NOS: ______-______), NM_080392 (SEQ ID NOS: ______-______), NM_080391 (SEQ ID NOS: ______-______), NM_032611 (SEQ ID NOS: ______-______), and NM_007079 (SEQ ID NOS: ______-______); SHP-2 (GenBank Accession Nos. D13540 (SEQ ID NOS: ______-______); L03535 (SEQ ID NOS: ______-______); L07527 (SEQ ID NOS: ______-______); X70766 (SEQ ID NOS: ______-______); L08807 (SEQ ID NO: ______); 78088 (SEQ ID NOS: ______-______); S39383 (SEQ ID NO: ______); D84372 (SEQ ID NOS: ______-______); U09307 (SEQ ID NOS: ______-______); CD45 (e.g., (Charbonneau et al., *Proc. Natl. Acad. Sci. USA* 85:7182-86 (1988); Genbank Accession Nos. NM_080922 (SEQ ID NOS: ______-______), NM_080921 (SEQ ID NOS: ______-______), NM_002838 (SEQ ID NOS: ______-______), and NM_080923) (SEQ ID NOS: ______-______); GenBank Acc. No. XM_16748; e.g., SEQ ID NO:32 encoded by SEQ ID NO:31); SEQ ID NOS:______-______); DSP-11 (WO 01/05983, SEQ ID NO:26 encoded by SEQ ID NO:25); DSP-18 (U.S. application Ser. No. 10/151,320, SEQ ID NO:28 encoded by SEQ ID NO:27); DSP-13 (U.S. application Ser. No. 09/775,925; SEQ ID NO:______ encoded by SEQ ID NO:______); DSP-14 (U.S. application Ser. No. 09/847,519; SEQ ID NO:______ encoded by SEQ ID NO:______); WO 01/46394); MKP-1 (WO 97/00315; Keyse et al., 1992 *Nature* 59:644; SEQ ID NO:30 encoded by SEQ ID NO:29). According to the contemplated invention, the siRNA polynucleotide expressly does not consist of a CDC14a.5 polynucleotide having a sequence set forth in SEQ ID NO:10 (Mailand et al., 2002 *Nature Cell Biol.* 4:317).

[0072] In certain embodiments of the invention, an siRNA polynucleotide interferes with expression of a component of a signaling transduction pathway, for example, components of the JNK signaling transduction pathway such as MKK4 (e.g., GenBank Accession Nos. L36870 (SEQ ID NO:______ and ______), NM_009157, and NM_009157; SEQ ID NO: ______ encoded by SEQ ID NO: ______)) and MKK7 (e.g., GenBank Accession Nos. AF013588 (SEQ ID NO: ______ encoded by SEQ ID NO:______) and AF026216, and to related compositions and methods. (See also Shen et al., *Proc. Natl. Acad. Sci. USA* 98:13613-18 (2001)). In certain other embodiments of the invention, the siRNA polynucleotide interferes with expression of a cellular polypeptide or enzyme that is associated with a cellular malfunction or defect (e.g., in a cancer or malignancy, an enzyme that is overexpressed or constitutively expressed and is associated with cell survival, proliferation, apoptosis, cell division, and differentiation). For example, the siRNA polynucleotide may comprise a sequence specific for dihydrofolate reductase (DHFR) (e.g., GenBank Accession No: NM_000791; SEQ ID NO: ______ encoded by SEQ ID NO:______)); thymidylate synthetase e.g., GenBank Accession No: NM_001071 (SEQ ID NO: ______ encoded by SEQ ID NO:______); topoisomerase I (e.g., GenBank Accession No: J03250; SEQ ID NO: ______ encoded by SEQ ID NO:______)); IkappaB kinase (IKK) alpha (e.g., GenBank Accession No. AF080157; SEQ ID NO: ______ encoded by SEQ ID NO:______); GenBank Accession No. AF009225; GenBank Accession No. AF012890); IKKbeta e.g., GenBank Accession No. AF080158; SEQ ID NO: ______ encoded by SEQ ID NO:______); GenBank Accession No. AF031416; GenBank Accession No. AF029684);

or IKKgamma e.g., GenBank Accession No. AF074382; SEQ ID NO: ______ encoded by SEQ ID NO:______); GenBank Accession No. AF091453).

[0073] In another preferred embodiment, the siRNA polynucleotides provided interfere with expression of DSP-3, SHP-2, CD45, PTPϵ, KAP, cdc14a, cdc14b, cdc25A, cdc25B, cdc25C, and PRL-3. According to non-limiting theory, the siRNA polynucleotides of the present invention direct sequence-specific degradation of mRNA encoding a PTP such as SHP2, PTPϵ, or a dual specificity phosphatase (e.g., DSP-3, KAP, cdc14a, cdc14b, cdc25A, cdc25B, cdc25C, CD45, or PRL-3) by a mechanism known as RNA interference (RNAi). The invention is not intended, however, to be so limited, and certain embodiments relate to RNA interference of other PTPs and dual specificity phosphatases (e.g., DSP-11, DSP-13, DSP-14, and DSP-18), and to interference with expression of other polypeptides and components of signal transduction pathways including mitogen activated protein (MAP) kinases, which include a MAP kinase kinase (e.g., MAPKKK or MEKK) that activates a MAP/ERK kinase (e.g., MAPKK or MEK), which then stimulates a phosphorylation-dependent increase in the activity of the MAP kinase. Upon activation, a MAP kinase can phosphorylate a variety of intracellular targets including transcription factors, transcriptional adaptor proteins, membrane and cytoplasmic substrates, and other protein kinases. In certain preferred embodiments, a siRNA polynucleotide interferes with expression of a MAP kinase kinase that is a component of the JNK signal transduction pathway, for example, MKK4 or MKK7. In other preferred embodiments, a siRNA polynucleotide interferes with expression of a cellular polypeptide or enzyme that is associated with a cellular malfunction or defect in cancer or malignancy, and which may be overexpressed or constitutively expressed in the tumor cell.

[0074] In addition, other preferred polypeptides include polypeptides that are targets of chemotherapeutic agents or drugs. Examples of chemotherapeutic target polypeptides include enzymes in the folate metabolic pathway, for example, thymidylate synthetase, which is a target of fluoropyrmidines. Another enzyme in this pathway is dihydrofolate reductase (DHFR), which is targeted by antifolate agents, such as methotrexate. DNA processing enzymes, including topoisomerase I and topoisomerase II, are also targets of chemotherapeutic agents. Other examples of chemotherapeutic target polypeptides include microtubule polypeptides, which are chemotherapeutic targets of taxanes and vinca alkaloids. According to non-limiting theory, these chemotherapeutic target polypeptides may become resistant to a drug or agent, that is, resistance may be manifested by overexpression or constitutive expression of the chemotherapeutic target polypeptide in a target cell. The overexpression of such a target polypeptide may be reduced by introducing a specific siRNA polynucleotide into the cell. In certain embodiments of the invention, a siRNA polynucleotide interferes with expression of such chemotherapeutic target polypeptides. For example, siRNA polynucleotides of the present invention that interfere with expression of a chemotherapeutic target polypeptide comprise sequences specific for dihydrofolate reductase (DHFR), thymidylate synthetase, topoisomerase I, and IKKgamma.

[0075] SiRNA Polynucleotides

[0076] As used herein, the term "siRNA" means either: (i) a double stranded RNA oligonucleotide, or polynucleotide, that is 18 base pairs, 19 base pairs, 20 base pairs, 21 base pairs, 22 base pairs, 23 base pairs, 24 base pairs, 25 base pairs, 26 base pairs, 27 base pairs, 28 base pairs, 29 base pairs or 30 base pairs in length and that is capable of interfering with expression and activity of a PTP-1B polypeptide, or a variant of the PTP-1B polypeptide, wherein a single strand of the siRNA comprises a portion of a RNA polynucleotide sequence that encodes the PTP-1B polypeptide, its variant, or a complementary sequence thereto; (ii) a single stranded oligonucleotide, or polynucleotide of 18 nucleotides, 19 nucleotides, 20 nucleotides, 21 nucleotides, 22 nucleotides, 23 nucleotides, 24 nucleotides, 25 nucleotides, 26 nucleotides, 27 nucleotides, 28 nucleotides, 29 nucleotides or 30 nucleotides in length and that is either capable of interfering with expression and/or activity of a target polypeptide such as DSP-3, SHP-2, KAP, PRL-3, cdc14 or cdc25, or a variant of the target polypeptide, or that anneals to a complementary sequence to result in a dsRNA that is capable of interfering with target polypeptide expression, wherein such single stranded oligonucleotide comprises a portion of a RNA polynucleotide sequence that encodes the target polypeptide, its variant, or a complementary sequence thereto; or (iii) an oligonucleotide, or polynucleotide, of either (i) or (ii) above wherein such oligonucleotide, or polynucleotide, has one, two, three or four nucleic acid alterations or substitutions therein.

[0077] A siRNA polynucleotide is a RNA nucleic acid molecule that mediates the effect of RNA interference, a post-transcriptional gene silencing mechanism. A siRNA polynucleotide preferably comprises a double-stranded RNA (dsRNA) but is not intended to be so limited and may comprise a single-stranded RNA (see, e.g., Martinez et al. *Cell* 110:563-74 (2002)). A siRNA polynucleotide may comprise other naturally occurring, recombinant, or synthetic single-stranded or double-stranded polymers of nucleotides (ribonucleotides or deoxyribonucleotides or a combination of both) and/or nucleotide analogues as provided herein (e.g., an oligonucleotide or polynucleotide or the like, typically in 5' to 3' phosphodiester linkage). Accordingly it will be appreciated that certain exemplary sequences disclosed herein as DNA sequences capable of directing the transcription of the subject invention siRNA polynucleotides are also intended to describe the corresponding RNA sequences and their complements, given the well established principles of complementary nucleotide base-pairing. A siRNA may be transcribed using as a template a DNA (genomic, cDNA, or synthetic) that contains a RNA polymerase promoter, for example, a U6 promoter or the H1 RNA polymerase III promoter, or the siRNA may be a synthetically derived RNA molecule. In certain embodiments the subject invention siRNA polynucleotide may have blunt ends, that is, each nucleotide in one strand of the duplex is perfectly complementary (e.g., by Watson-Crick base-pairing) with a nucleotide of the opposite strand. In certain other embodiments, at least one strand of the subject invention siRNA polynucleotide has at least one, and preferably two nucleotides that "overhang" (i.e., that do not base pair with a complementary base in the opposing strand) at the 3' end of either strand, or preferably both strands, of the siRNA polynucleotide. In a preferred embodiment of the invention, each strand of the siRNA polynucleotide duplex

has a two-nucleotide overhang at the 3' end. The two-nucleotide overhang is preferably a thymidine dinucleotide (TT) but may also comprise other bases, for example, a TC dinucleotide or a TG dinucleotide, or any other dinucleotide. The overhang dinucleotide may also be complementary to the two nucleotides at the 5' end of the sequence of the polynucleotide that is targeted for interference. For a discussion of 3' ends of siRNA polynucleotides see, e.g., WO 01/75164.

[0078] Preferred siRNA polynucleotides comprise double-stranded oligomeric nucleotides of about 18-30 nucleotide base pairs, preferably about 18, 19, 20, 21, 22, 23, 24, 25, 26, or 27 base pairs, and in other preferred embodiments about 19, 20, 21, 22 or 23 base pairs, or about 27 base pairs, whereby the use of "about" indicates, as described above, that in certain embodiments and under certain conditions the processive cleavage steps that may give rise to functional siRNA polynucleotides that are capable of interfering with expression of a selected polypeptide may not be absolutely efficient. Hence, siRNA polynucleotides, for instance, of "about" 18, 19, 20, 21, 22, 23, 24, or 25 base pairs may include one or more siRNA polynucleotide molecules that may differ (e.g., by nucleotide insertion or deletion) in length by one, two, three or four base pairs, by way of non-limiting theory as a consequence of variability in processing, in biosynthesis, or in artificial synthesis. The contemplated siRNA polynucleotides of the present invention may also comprise a polynucleotide sequence that exhibits variability by differing (e.g., by nucleotide substitution, including transition or transversion) at one, two, three or four nucleotides from a particular sequence, the differences occurring at any of positions 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, or 19 of a particular siRNA polynucleotide sequence, or at positions 20, 21, 22, 23, 24, 25, 26, or 27 of siRNA polynucleotides depending on the length of the molecule, whether situated in a sense or in an antisense strand of the double-stranded polynucleotide. The nucleotide substitution may be found only in one strand, by way of example in the antisense strand, of a double-stranded polynucleotide, and the complementary nucleotide with which the substitute nucleotide would typically form hydrogen bond base pairing may not necessarily be correspondingly substituted in the sense strand. In preferred embodiments, the siRNA polynucleotides are homogeneous with respect to a specific nucleotide sequence. As described herein, preferred siRNA polynucleotides interfere with expression of a DSP-3, SHP-2, KAP, PRL-3, cdc14 or cdc25 polypeptide. These polynucleotides may also find uses as probes or primers.

[0079] Polynucleotides that are siRNA polynucleotides of the present invention may in certain embodiments be derived from a single-stranded polynucleotide that comprises a single-stranded oligonucleotide fragment (e.g., of about 18-30 nucleotides, which should be understood to include any whole integer of nucleotides including and between 18 and 30) and its reverse complement, typically separated by a spacer sequence. According to certain such embodiments, cleavage of the spacer provides the single-stranded oligonucleotide fragment and its reverse complement, such that they may anneal to form (optionally with additional processing steps that may result in addition or removal of one, two, three or more nucleotides from the 3' end and/or the 5' end of either or both strands) the double-stranded siRNA polynucleotide of the present invention. In certain embodiments the spacer is of a length that permits the fragment and its reverse complement to anneal and form a double-stranded structure (e.g., like a hairpin polynucleotide) prior to cleavage of the spacer (and, optionally, subsequent processing steps that may result in addition or removal of one, two, three, four, or more nucleotides from the 3' end and/or the 5' end of either or both strands). A spacer sequence may therefore be any polynucleotide sequence as provided herein that is situated between two complementary polynucleotide sequence regions which, when annealed into a double-stranded nucleic acid, comprise a siRNA polynucleotide. Preferably a spacer sequence comprises at least 4 nucleotides, although in certain embodiments the spacer may comprise 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21-25, 26-30, 31-40, 41-50, 51-70, 71-90, 91-110, 111-150, 151-200 or more nucleotides. Examples of siRNA polynucleotides derived from a single nucleotide strand comprising two complementary nucleotide sequences separated by a spacer have been described (e.g., Brummelkamp et al., 2002 *Science* 296:550; Paddison et al., 2002 *Genes Develop.* 16:948; Paul et al. *Nat. Biotechnol.* 20:505-508 (2002); Grabarek et al., *BioTechniques* 34:734-44 (2003)).

[0080] Polynucleotide variants may contain one or more substitutions, additions, deletions, and/or insertions such that the activity of the siRNA polynucleotide is not substantially diminished, as described above. The effect on the activity of the siRNA polynucleotide may generally be assessed as described herein, or using conventional methods. Variants preferably exhibit at least about 75%, 78%, 80%, 85%, 87%, 88% or 89% identity and more preferably at least about 90%, 92%, 95%, 96%, or 97% identity to a portion of a polynucleotide sequence that encodes a native DSP-3, SHP-2, KAP, PRL-3, cdc14 or cdc25. The percent identity may be readily determined by comparing sequences of the polynucleotides to the corresponding portion of the target polynucleotide, using any method including using computer algorithms well known to those having ordinary skill in the art, such as Align or the BLAST algorithm (Altschul, *J. Mol. Biol.* 219:555-565, 1991; Henikoff and Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915-10919, 1992), which is available at the NCBI website (see [online] Internet:<URL:http://www/ncbi.nlm.nih.gov/cgi-bin/BLAST). Default parameters may be used.

[0081] Certain siRNA polynucleotide variants are substantially homologous to a portion of a native gene that encodes a desired target polypeptide. Single-stranded nucleic acids derived (e.g., by thermal denaturation) from such polynucleotide variants are capable of hybridizing under moderately stringent conditions to a naturally occurring DNA or RNA sequence encoding a native target polypeptide. In a preferred embodiment of the invention, a siRNA polynucleotide that detectably hybridizes under moderately stringent conditions to a target polypeptide-encoding polynucleotide comprises a nucleotide sequence other than SEQ ID NO:10, which is disclosed in Mailand et al. (2002 *Nature Cell Biol.* 4:317). A siRNA polynucleotide that detectably hybridizes under moderately stringent conditions may have a nucleotide sequence that includes at least 10 consecutive nucleotides, more preferably 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 consecutive nucleotides that are complementary to a particular target polynucleotide. In certain preferred embodiments such a siRNA sequence (or its complement) will be unique to a single particular target

polypeptide for which interference with expression is desired, and in certain other embodiments the sequence (or its complement) may be shared by two or more related target polypeptides for which interference with polypeptide expression is desired.

[0082] Suitable moderately stringent conditions include, for example, pre-washing in a solution of 5×SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0); hybridizing at 50° C.-70° C., 5×SSC for 1-16 hours (e.g., overnight); followed by washing once or twice at 22-65° C. for 20-40 minutes with one or more each of 2×, 0.5× and 0.2×SSC containing 0.05-0.1% SDS. For additional stringency, conditions may include a wash in 0.1×SSC and 0.1% SDS at 50-60° C. for 15-40 minutes. As known to those having ordinary skill in the art, variations in stringency of hybridization conditions may be achieved by altering the time, temperature, and/or concentration of the solutions used for pre-hybridization, hybridization, and wash steps. Suitable conditions may also depend in part on the particular nucleotide sequences of the probe used, and of the blotted, proband nucleic acid sample. Accordingly, it will be appreciated that suitably stringent conditions can be readily selected without undue experimentation when a desired selectivity of the probe is identified, based on its ability to hybridize to one or more certain proband sequences while not hybridizing to certain other proband sequences.

[0083] Sequence specific siRNA polynucleotides of the present invention may be designed using one or more of several criteria. For example, to design a siRNA polynucleotide that has 19 consecutive nucleotides identical to a sequence encoding a polypeptide of interest (e.g., PTP1B and other polypeptides described herein), the open reading frame of the polynucleotide sequence may be scanned for 21-base sequences that have one or more of the following characteristics: (1) an A+T/G+C ratio of approximately 1:1 but no greater than 2:1 or 1:2; (2) an AA dinucleotide or a CA dinucleotide at the 5' end; (3) an internal hairpin loop melting temperature less than 55° C.; (4) a homodimer melting temperature of less than 37° C. (melting temperature calculations as described in (3) and (4) can be determined using computer software known to those skilled in the art); (5) a sequence of at least 16 consecutive nucleotides not identified as being present in any other known polynucleotide sequence (such an evaluation can be readily determined using computer programs available to a skilled artisan such as BLAST to search publicly available databases). Alternatively, a siRNA polynculeotide sequence may be designed and chosen using a computer software available commercially from various vendors (e.g., OligoEngine™ (Seattle, Wash.); Dharmacon, Inc. (Lafayette, Colo.); Ambion Inc. (Austin, Tex.); and QIAGEN, Inc. (Valencia, Calif.)). (See also Elbashir et al., *Genes & Development* 15:188-200 (2000); Elbashir et al., *Nature* 411:494-98 (2001); and [online] Internet:URL<http://www.mpibpc.gwdg.de/abteilungen/100/105/Tusch1_MIV2(3) 2002.pdf.) The siRNA polynucleotides may then be tested for their ability to interfere with the expression of the target polypeptide according to methods known in the art and described herein. The determination of the effectiveness of an siRNA polynucleotide includes not only consideration of its ability to interfere with polypeptide expression but also includes consideration of whether the siRNA polynucleotide manifests undesirably toxic effects, for example, apoptosis of a cell for which cell death is not a desired effect of RNA interference (e.g., interference of PTP1B expression in a cell).

[0084] It should be appreciated that not all siRNAs designed using the above methods will be effective at silencing or interfering with expression of a desired target polypeptide. And further, that the siRNAs will effect silencing to different degrees. Such siRNAs must be tested for their effectiveness, and selections made therefrom based on the ability of a given siRNA to interfere with or modulate (e.g., decrease in a statistically significant manner) the expression of the target. Accordingly, identification of specific siRNA polynucleotide sequences that are capable of interfering with expression of a desired target polypeptide requires production and testing of each siRNA, as demonstrated in greater detail below (see Examples).

[0085] Furthermore, not all siRNAs that interfere with protein expression will have a physiologically important effect. The inventors here have designed, and describe herein, physiologically relevant assays for measuring the influence of modulated target polypeptide expression, for instance, cellular proliferation, induction of apoptosis, and/or altered levels of protein tyrosine phosphorylation (e.g., insulin receptor phosphorylation), to determine if the levels of interference with target protein expression that were observed using the siRNAs of the invention have clinically relevant significance. Additionally, and according to non-limiting theory, the invention contemplates altered (e.g., decreased or increased in a statistically significant manner) expression levels of one or more polypeptides of interest, and/or altered (i.e., increased or decreased) phosphorylation levels of one or more phosphoproteins of interest, which altered levels may result from impairment of target protein expression and/or cellular compensatory mechanisms that are induced in response to RNAi-mediated inhibition of a specific target polypeptide expression.

[0086] Persons having ordinary skill in the art will also readily appreciate that as a result of the degeneracy of the genetic code, many nucleotide sequences may encode a polypeptide as described herein. That is, an amino acid may be encoded by one of several different codons and a person skilled in the art can readily determine that while one particular nucleotide sequence may differ from another (which may be determined by alignment methods disclosed herein and known in the art), the sequences may encode polypeptides with identical amino acid sequences. By way of example, the amino acid leucine in a polypeptide may be encoded by one of six different codons (TTA, TTG, CTT, CTC, CTA, and CTG) as can serine (TCT, TCC, TCA, TCG, AGT, and AGC). Other amino acids, such as proline, alanine, and valine, for example, may be encoded by any one of four different codons (CCT, CCC, CCA, CCG for proline; GCT, GCC, GCA, GCG for alanine; and GTT, GTC, GTA, GTG for valine). Some of these polynucleotides bear minimal homology to the nucleotide sequence of any native gene. Nonetheless, polynucleotides that vary due to differences in codon usage are specifically contemplated by the present invention.

[0087] Polynucleotides, including target polynucleotides, may be prepared using any of a variety of techniques, which will be useful for the preparation of specifically desired siRNA polynucleotides and for the identification and selec-

tion of desirable sequences to be used in siRNA polynucleotides. For example, a polynucleotide may be amplified from cDNA prepared from a suitable cell or tissue type. Such polynucleotides may be amplified via polymerase chain reaction (PCR). For this approach, sequence-specific primers may be designed based on the sequences provided herein and may be purchased or synthesized. An amplified portion may be used to isolate a full-length gene, or a desired portion thereof, from a suitable library (e.g., human skeletal muscle cDNA) using well known techniques. Within such techniques, a library (cDNA or genomic) is screened using one or more polynucleotide probes or primers suitable for amplification. Preferably, a library is size-selected to include larger molecules. Random primed libraries may also be preferred for identifying 5' and upstream regions of genes. Genomic libraries are preferred for obtaining introns and extending 5' sequences. Suitable sequences for a siRNA polynucleotide contemplated by the present invention may also be selected from a library of siRNA polynucleotide sequences.

[0088] For hybridization techniques, a partial sequence may be labeled (e.g., by nick-translation or end-labeling with ^{32}P) using well known techniques. A bacterial or bacteriophage library may then be screened by hybridizing filters containing denatured bacterial colonies (or lawns containing phage plaques) with the labeled probe (see, e.g., Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, N.Y., 2001). Hybridizing colonies or plaques are selected and expanded, and the DNA is isolated for further analysis. Clones may be analyzed to determine the amount of additional sequence by, for example, PCR using a primer from the partial sequence and a primer from the vector. Restriction maps and partial sequences may be generated to identify one or more overlapping clones. A full-length cDNA molecule can be generated by ligating suitable fragments, using well known techniques.

[0089] Alternatively, numerous amplification techniques are known in the art for obtaining a full-length coding sequence from a partial cDNA sequence. Within such techniques, amplification is generally performed via PCR. One such technique is known as "rapid amplification of cDNA ends" or RACE. This technique involves the use of an internal primer and an external primer, which hybridizes to a polyA region or vector sequence, to identify sequences that are 5' and 3' of a known sequence. Any of a variety of commercially available kits may be used to perform the amplification step. Primers may be designed using, for example, software well known in the art. Primers (or oligonucleotides for other uses contemplated herein, including, for example, probes and antisense oligonucleotides) are preferably 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31 or 32 nucleotides in length, have a GC content of at least 40% and anneal to the target sequence at temperatures of about 54° C. to 72° C. The amplified region may be sequenced as described above, and overlapping sequences assembled into a contiguous sequence. Certain oligonucleotides contemplated by the present invention may, for some preferred embodiments, have lengths of 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33-35, 35-40, 41-45, 46-50, 56-60, 61-70, 71-80, 81-90 or more nucleotides.

[0090] A number of specific siRNA polynucleotide sequences useful for interfering with target polypeptide expression, and are presented in the Examples, the Drawings, and the Sequence Listing. SiRNA polynucleotides may generally be prepared by any method known in the art, including, for example, solid phase chemical synthesis. Modifications in a polynucleotide sequence may also be introduced using standard mutagenesis techniques, such as oligonucleotide-directed site-specific mutagenesis. Further, siRNAs may be chemically modified or conjugated to improve their serum stability and/or delivery properties. Included as an aspect of the invention are the siRNAs described herein wherein the ribose has been removed therefrom. Alternatively, siRNA polynucleotide molecules may be generated by in vitro or in vivo transcription of suitable DNA sequences (e.g., polynucleotide sequences encoding a target polypeptide, or a desired portion thereof), provided that the DNA is incorporated into a vector with a suitable RNA polymerase promoter (such as T7, U6, H1, or SP6). In addition, a siRNA polynucleotide may be administered to a patient, as may be a DNA sequence (e.g., a recombinant nucleic acid construct as provided herein) that supports transcription (and optionally appropriate processing steps) such that a desired siRNA is generated in vivo.

[0091] Accordingly, a siRNA polynucleotide that is complementary to at least a portion of a target polypeptide-encoding sequence may be used to modulate gene expression, or as a probe or primer. Identification of siRNA polynucleotide sequences and DNA encoding genes for their targeted delivery involves techniques described herein. Identification of such siRNA polynucleotide sequences and DNA encoding genes for their targeted delivery involves techniques that are also described herein. As discussed above, siRNA polynucleotides exhibit desirable stability characteristics and may, but need not, be further designed to resist degradation by endogenous nucleolytic enzymes by using such linkages as phosphorothioate, methylphosphonate, sulfone, sulfate, ketyl, phosphorodithioate, phosphoramidate, phosphate esters, and other such linkages (see, e.g., Agrwal et al., *Tetrahedron Lett.* 28:3539-3542 (1987); Miller et al., *J. Am. Chem. Soc.* 93:6657-6665 (1971); Stec et al., *Tetrahedron Lett.* 26:2191-2194 (1985); Moody et al., *Nucleic Acids Res.* 12:4769-4782 (1989); Uznanski et al., *Nucleic Acids Res.* (1989); Letsinger et al., *Tetrahedron* 40:137-143 (1984); Eckstein, *Annu. Rev. Biochem.* 54:367402 (1985); Eckstein, *Trends Biol. Sci.* 14:97-100 (1989); Stein, In: *Oligodeoxynucleotides. Antisense Inhibitors of Gene Expression*, Cohen, ed., Macmillan Press, London, pp. 97-117 (1989); Jager et al., *Biochemistry* 27:7237-7246 (1988)).

[0092] Any polynucleotide of the invention may be further modified to increase stability in vivo. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends; the use of phosphorothioate or 2' O-methyl rather than phosphodiester linkages in the backbone; and/or the inclusion of nontraditional bases such as inosine, queosine, and wybutosine and the like, as well as acetyl- methyl-, thio- and other modified forms of adenine, cytidine, guanine, thymine, and uridine.

[0093] Nucleotide sequences as described herein may be joined to a variety of other nucleotide sequences using established recombinant DNA techniques. For example, a polynucleotide may be cloned into any of a variety of

cloning vectors, including plasmids, phagemids, lambda phage derivatives, and cosmids. Vectors of particular interest include expression vectors, replication vectors, probe generation vectors, and sequencing vectors. In general, a suitable vector contains an origin of replication functional in at least one organism, convenient restriction endonuclease sites, and one or more selectable markers. (See, e.g., WO 01/96584; WO 01/29058; U.S. Pat. No. 6,326,193; U.S. 2002/0007051). Other elements will depend upon the desired use, and will be apparent to those having ordinary skill in the art. For example, the invention contemplates the use of siRNA polynucleotide sequences in the preparation of recombinant nucleic acid constructs including vectors for interfering with the expression of a desired target polypeptide such as a PTP polypeptide, a MAP kinase kinase polypeptide, or a chemotherapeutic target polypeptide in vivo; the invention also contemplates the generation of siRNA transgenic or "knock-out" animals and cells (e.g., cells, cell clones, lines or lineages, or organisms in which expression of one or more desired polypeptides (e.g., a target polypeptide) is fully or partially compromised). An siRNA polynucleotide that is capable of interfering with expression of a desired polypeptide (e.g., a target polypeptide) as provided herein thus includes any siRNA polynucleotide that, when contacted with a subject or biological source as provided herein under conditions and for a time sufficient for target polypeptide expression to take place in the absence of the siRNA polynucleotide, results in a statistically significant decrease (alternatively referred to as "knockdown" of expression) in the level of target polypeptide expression that can be detected. Preferably the decrease is greater than 10%, more preferably greater than 20%, more preferably greater than 30%, more preferably greater than 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95% or 98% relative to the expression level of the polypeptide detected in the absence of the siRNA, using conventional methods for determining polypeptide expression as known to the art and provided herein. Preferably, the presence of the siRNA polynucleotide in a cell does not result in or cause any undesired toxic effects, for example, apoptosis or death of a cell in which apoptosis is not a desired effect of RNA interference.

[0094] Within certain embodiments, siRNA polynucleotides may be formulated so as to permit entry into a cell of a mammal, and expression therein. Such formulations are particularly useful for therapeutic purposes, as described below. Those having ordinary skill in the art will appreciate that there are many ways to achieve expression of a polynucleotide in a target cell, and any suitable method may be employed. For example, a polynucleotide may be incorporated into a viral vector using well known techniques (see also, e.g., U.S. 2003/0068821). A viral vector may additionally transfer or incorporate a gene for a selectable marker (to aid in the identification or selection of transduced cells) and/or a targeting moiety, such as a gene that encodes a ligand for a receptor on a specific target cell, to render the vector target specific. Targeting may also be accomplished using an antibody, by methods known to those having ordinary skill in the art.

[0095] Other formulations for therapeutic purposes include colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. A preferred colloidal system for use as a delivery vehicle in vitro and in vivo is a liposome (i.e., an artificial membrane vesicle). The preparation and use of such systems is well known in the art.

[0096] Within other embodiments, one or more promoters may be identified, isolated and/or incorporated into recombinant nucleic acid constructs of the present invention, using standard techniques. The present invention provides nucleic acid molecules comprising such a promoter sequence or one or more cis- or trans-acting regulatory elements thereof. Such regulatory elements may enhance or suppress expression of a siRNA. A 5' flanking region may be generated using standard techniques, based on the genomic sequence provided herein. If necessary, additional 5' sequences may be generated using PCR-based or other standard methods. The 5' region may be subcloned and sequenced using standard methods. Primer extension and/or RNase protection analyses may be used to verify the transcriptional start site deduced from the cDNA.

[0097] To define the boundary of the promoter region, putative promoter inserts of varying sizes may be subcloned into a heterologous expression system containing a suitable reporter gene without a promoter or enhancer. Suitable reporter genes may include genes encoding luciferase, beta-galactosidase, chloramphenicol acetyl transferase, secreted alkaline phosphatase, or the Green Fluorescent Protein gene (see, e.g., Ui-Tei et al., *FEBS Lett.* 479:79-82 (2000). Suitable expression systems are well known and may be prepared using well known techniques or obtained commercially. Internal deletion constructs may be generated using unique internal restriction sites or by partial digestion of non-unique restriction sites. Constructs may then be transfected into cells that display high levels of siRNA polynucleotide and/or polypeptide expression. In general, the construct with the minimal 5' flanking region showing the highest level of expression of reporter gene is identified as the promoter. Such promoter regions may be linked to a reporter gene and used to evaluate agents for the ability to modulate promoter-driven transcription.

[0098] Once a functional promoter is identified, cis- and trans-acting elements may be located. Cis-acting sequences may generally be identified based on homology to previously characterized transcriptional motifs. Point mutations may then be generated within the identified sequences to evaluate the regulatory role of such sequences. Such mutations may be generated using site-specific mutagenesis techniques or a PCR-based strategy. The altered promoter is then cloned into a reporter gene expression vector, as described above, and the effect of the mutation on reporter gene expression is evaluated.

[0099] In general, polypeptides and polynucleotides as described herein are isolated. An "isolated" polypeptide or polynucleotide is one that is removed from its original environment. For example, a naturally occurring protein is isolated if it is separated from some or all of the coexisting materials in the natural system. Preferably, such polypeptides are at least about 90% pure, more preferably at least about 95% pure and most preferably at least about 99% pure. A polynucleotide is considered to be isolated if, for example, it is cloned into a vector that is not a part of the natural environment. A "gene" includes the segment of DNA involved in producing a polypeptide chain; it further includes regions preceding and following the coding region "leader and trailer," for example promoter and/or enhancer

and/or other regulatory sequences and the like, as well as intervening sequences (introns) between individual coding segments (exons).

[0100] As noted above, according to certain embodiments of the invention compositions and methods are provided that relate to altering or altered expression of a PTP as described herein (including DSPs) or of other target polypeptides as disclosed herein, and/or to a PTP associated disorder. A PTP associated disorder includes any disease, disorder, condition, syndrome, pathologic or physiologic state, or the like, wherein at least one undesirable deviation or departure from a physiological norm causes, correlates with, is accompanied by or results from an inappropriate alteration (i.e., a statistically significant change) to the structure, activity, function, expression level, physicochemical or hydrodynamic property, or stability of a PTP or of a molecular component of a biological signal transduction pathway that comprises a PTP, for instance, a MAP kinase such as JNK (e.g., Shen et al., 2001 *Proc. Nat. Acad. Sci. USA* 98:13613; see also U.S. Pat. No. 6,342,595), TYK2 or Jak2 (e.g., Myers et al., 2001 *J. Biol. Chem.* 276:47771), or a MAP kinase kinase MKK4 or MKK7 (e.g., Shen et al., *Proc. Natl. Acad. Sci. USA* 98:13613-18 (2001) and references cited therein), a receptor such as IR (Salmeen et al., 2000), or leptin receptor (e.g., Kalman et al. 2000 and references cited therein) or other such pathways comprising PTPs as known to the art. In preferred embodiments the molecular component may be a protein, peptide or polypeptide, and in certain other preferred embodiments the alteration may be an altered level of PTP expression. In certain other preferred embodiments the alteration may be manifest as an a typical or unusual phosphorylation state of a protein under particular conditions, for example, hypophosphorylation or hyperphosphorylation of a phosphoprotein, wherein those familiar with the art will appreciate that phosphorylated proteins typically comprise one or more phosphotyrosine, phosphoserine, or phosphothreonine residues.

[0101] PTP associated disorders therefore include, for example, diabetes mellitus, obesity, impaired glucose tolerance and other metabolic disorders wherein alteration of a biological signaling pathway component is associated with the disorder The effect of siRNA interference with expression of a component in the signal transduction pathway induced by insulin, for example, may be evaluated by determining the level of tyrosine phosphorylation of insulin receptor beta (IR-β) and/or of the downstream signaling molecule PKB/Akt and/or of any other downstream polypeptide that may be a component of a particular signal transduction pathway as provided herein. The invention is not intended, however, to be so limited and contemplates other disorders, such as JNK-associated disorders (e.g., cancer, cardiac hypertrophy, ischemia, diabetes, hyperglycemia-induced apoptosis, inflammation, neurodegenerative disorders), and other disorders associated with different signal transduction pathways, for instance, cancer, autoimmunity, cellular proliferative disorders, neurodegenerative disorders, and infectious diseases (see, e.g., Fukada et al., 2001 *J. Biol. Chem.* 276:25512; Tonks et al., 2001 *Curr. Opin. Cell Biol.* 13:182; Salmeen et al., 2000 *Mol. Cell* 6:1401; Hu et al., *J. Neurochem.* 85:432-42 (2003); and references cited therein).

[0102] Cancer is also associated with other dual specificity phosphatases, such as DSP-3, PRL-3 (see, e.g., Saha et al., *Science* 294:1343-46 (2001), PTPϵ (Elson, *Oncogene* 18:7535-42 (1999)), and the cell cycle dual specificity phosphatases cdc25 (see, e.g., Donzelli et al., *EMBO* 21:4875-84 (2002), cdc14 (Wong et al., *Genomics* 59:248-51 (1999)), and KAP (see, e.g., Lee et al., *Mol. Cell Biol.* 20:1723-32 (2000); Yeh et al., *Cancer Res.* 60:4697-700 (2000); see also, e.g., Donato et al., *J. Clin. Invest.* 109:51-58 (2002)). Another dual specificity phosphatase believed to be involved in the cell cycle, cdc14, is reported to interact with the tumor suppressor protein p53 (Li et al., *J. Biol. Chem.* 275:2410014 (2000); see also Agami et al., *Cell* 102:55-66 (2000)). In normal cells, cdc14 is reported to be a part of the mitotic exit network, which involves intricate regulatory pathways that coordinate chromosome segregation and mitotic exit with physical separation of two nascent cells, and in cytokineses (see, e.g., Gruneberg et al., *J. Cell Biol.* 158:901-14 (2002); Trautman et al., *Curr. Biol.* 12:R733-R735 (2002); Visintin et al., *Mol. Cell* 2:709-18 (1998); see also Mailand et al., supra). Persons skilled in the art will be familiar with an array of criteria according to which it may be recognized what are, for instance, biological, physiological, pathological and/or clinical signs and/or symptoms of PTP associated and other disorders as provided herein (see, e.g., Irie-Sasaki et al., *Curr. Top. Med. Chem.* 3:783-96 (2003) (discussing role of CD45 in signal transduction pathways); Oh et al., *Mol. Cell Biol.* 19:3205-15 (1999) (describing regulation of early events in integrin signaling by SHP-2); Musante et al., *Eur. J. Hum. Genet.* 11:201-206 (2003), Tartaglia et al., *Nat. Genet.* 29:465-68 (2001), and Ion et al., *Hum. Genet.* 111:421-27 (2002) (discussing correlation between mutations in the PTPN11 gene that encodes SHP-2 and Noonan Syndrome)); Tanuma et al., *Blood* 98:3030-34 (2001) (reporting that PTPϵ inhibits IL-6 and IL-10 induced JAK-STAT signaling)).

[0103] Also contemplated by the invention are disorders associated with the NF-kappaB signaling pathway, for example, in cancer cells in which NF-kappaB is overexpressed or constitutively activated (see, e.g., Bayon et al., *Mol. Cell Biol.* 23:1061-74 (2003); Arsura et al., *Oncogene* 22:412-25 (2003)). Other disorders associated with the NF-kappaB signaling pathway include those associated with other components of the pathway, for example, inflammation associated with IkappaB kinase gamma (IKKgamma), which is an upstream regulator of NF-kappaB that is required for NF-kappaB activation by various stimuli (see, e.g., Makris et al., *Mol. Cell Biol.* 22:6573-81 (2002); Li et al., *J. Biol. Chem.* 277:45129-40 (2002); Sadikot et al., *J. Immunol.* 170:1091-98 (2003)).

[0104] As noted above, regulated tyrosine phosphorylation contributes to specific pathways for biological signal transduction, including those associated with cell division, cell survival, apoptosis, proliferation and differentiation, and "biological signal transduction pathways," or "inducible signaling pathways" in the context of the present invention include transient or stable associations or interactions among molecular components involved in the control of these and similar processes in cells. Depending on the particular pathway of interest, an appropriate parameter for determining induction of such pathway may be selected. For example, for signaling pathways associated with cell proliferation, a variety of well known methodologies are available for quantifying proliferation, including, for example, incorporation of tritiated thymidine into cellular DNA, monitoring of detectable (e.g., fluorimetric or calorimetric)

indicators of cellular respiratory activity (for example, conversion of the tetrazolium salts (yellow) 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) or 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium (MTS) to formazan dyes (purple) in metabolically active cells), or cell counting, or the like. Similarly, in the cell biology arts, multiple techniques are known for assessing cell survival (e.g., vital dyes, metabolic indicators, etc.) and for determining apoptosis (for example, annexin V binding, DNA fragmentation assays, caspase activation, marker analysis, e.g., poly(ADP-ribose) polymerase (PARP), etc.). Other signaling pathways will be associated with particular cellular phenotypes, for example specific induction of gene expression (e.g., detectable as transcription or translation products, or by bioassays of such products, or as nuclear localization of cytoplasmic factors), altered (e.g., statistically significant increases or decreases) levels of intracellular mediators (e.g., activated kinases or phosphatases, altered levels of cyclic nucleotides or of physiologically active ionic species, etc.), altered cell cycle profiles, or altered cellular morphology, and the like, such that cellular responsiveness to a particular stimulus as provided herein can be readily identified to determine whether a particular cell comprises an inducible signaling pathway.

[0105] In addition, according to certain embodiments of the invention compositions and methods are provided that relate to altering or altered expression of chemotherapeutic target polypeptides. Sequence specific siRNA polynucleotides may be used as a conjunctive therapy with chemotherapeutic drugs or may provide an alternative therapy in circumstances when a cancer becomes refractory to chemotherapeutic treatment regimens. Resistance to chemotherapeutic drugs may develop when a chemotherapeutic target polypeptide is overexpressed or when its expression becomes constitutive. Overexpression or amplified expression of such a target polypeptide could be reduced by introducing a specific siRNA polynucleotide into the cell. In particular, chemotherapeutic target polypeptides that may become resistant to drug therapies include, for example, components of the thymidylate biosynthesis pathway, thymidylate synthetase and DHFR, which become refractory to anti-neoplastic drugs such as 5-FU and methotrexate, respectively, and contribute to a drug resistance phenotype. Also contemplated by the invention are sequence specific siRNA polynucleotides that interfere with expression of DNA-processing enzymes such as topoisomerase I and that would have anti-cancer or anti-bacterial effects. The effect of siRNA interference on expression of such chemotherapeutic target polypeptides may alter cell division, cell survival, apoptosis, proliferation, and differentiation, which may be assessed by any of the techniques and methods described herein.

[0106] PTPs

[0107] As used herein, a phosphatase is a member of the PTP family if it contains the signature motif CX_5R (SEQ ID NO:______). Dual specificity PTPs, i.e., PTPs that dephosphorylate both phosphorylated tyrosine and phosphorylated serine or threonine, are also suitable for use in the invention. PTPs for use in the present invention include PTP1B (e.g., GenBank Accession Nos. M31724 (SEQ ID NOS: ______-______); NM_002827 (SEQ ID NOS: ______-______); NM_011201 (SEQ ID NOS: ______-______); M31724 (SEQ ID NOS: ______-______); M33689 (SEQ ID NOS: ______-______); M33962 (SEQ ID NOS: ______-______)). In certain preferred embodiments, TC-PTP (e.g., GenBank Accession Nos. M25393 (SEQ ID NOS: ______-______); M81478 (SEQ ID NO: ______); M80737 (SEQ ID NO: ______); M81477 (SEQ ID NOS: ______-______); X58828 (SEQ ID NOS: ______-______); NM_002828 (SEQ ID NOS: ______ and ______)) and TC45 (e.g., NM_080422 (SEQ ID NOS: ______ and ______)) may be used. In certain other embodiments PTPs and DSPs for use in the present invention include DSP-3 (WO00/60092); SHP2, (e.g., GenBank Accession Nos. D13540 (SEQ ID NOS: ______-______); L03535 (SEQ ID NOS: ______-______); L07527 (SEQ ID NOS: ______-______); X70766 (SEQ ID NOS: ______-______); L08807 (SEQ ID NO: ______); S78088 (SEQ ID NOS: ______-______); S39383 (SEQ ID NO: ______); D84372 (SEQ ID NOS: ______-______); U09307 (SEQ ID NOS: 15-16)); cdc14 (which includes cdc14a (e.g., GenBank Accession Nos. AF122013 (SEQ ID NOS: ______-______); AF064102 (SEQ ID NOS: ______-______); AF064103 (SEQ ID NOS: ______-______); Li et al., 1997 *J. Biol. Chem.* 272:29403; U.S. Pat. No. 6,331,614) and cdc14b (e.g., GenBank Accession Nos. AF064104 (SEQ ID NOS: ______-______); AF064105 (SEQ ID NOS: ______-______); CDC25A ((e.g., GenBank Accession Nos. NM_001789 (SEQ ID NOS: ______-______), AF527417 (SEQ ID NOS: ______-______), NM_133571 (SEQ ID NOS: ______-______)); CDC25B (e.g., GenBank Accession Nos. NM_133572 (SEQ ID NOS: ______-______), NM_023117 (SEQ ID NOS: ______-______), NM_021872 (SEQ ID NOS: ______-______); NM_021872; M81934) (SEQ ID NOS: ______-______); and CDC25C (e.g., GenBank Accession Nos. NM_001790 (SEQ ID NOS: ______-______), NM_022809 (SEQ ID NOS: ______-______)); CD45 (Charbonneau et al., *Proc. Natl. Acad. Sci. USA* 85:7182-86 (1988); Genbank Accession Nos. NM_080922 (SEQ ID NOS: ______-______), NM_080921 (SEQ ID NOS: ______-______), NM_002838 (SEQ ID NOS: ______-______), and NM_080923) (SEQ ID NOS: ______-______); GenBank Acc. No. XM_16748; SEQ ID NO:32 encoded by SEQ ID NO:31; KAP (Genbank Accession No. L27711 (SEQ ID NOS: ______-______); Hannon et al., *Proc. Natl. Acad. Sci. USA* 91:1731-35 (1994)); PTPϵ (e.g., Genbank Accession Nos. NM_006504 (SEQ ID NOS: ______-______) and NM_130435 (SEQ ID NOS: ______-______)); and PRL-3 (e.g., Zhao et al., *Genomics* 35:172-81 (1996); Genbank Accession Nos. (NM 003479 (SEQ ID NOS: ______-______), NM_080392 (SEQ ID NOS: ______-______), NM_080391 (SEQ ID NOS: ______-______), NM_032611 (SEQ ID NOS: ______-______), and NM_007079 (SEQ ID NOS: ______-______). In certain preferred embodiments PTPs and DSPs include, but are not limited to, U.S. application Ser. No. 10/151,320 (DSP18); WO 01/05983 (DSP-11); U.S. application Ser. No. 09/775,925 (DSP-12 and DSP-13); U.S. application Ser. No. 09/847,519 and WO 01/46394 (DSP-14); The invention also contemplates using mutated forms of the PTPs and DSPs, which may include PTPs and DSPs that contain single nucleotide polymorphisms (SNPs), or may include allelic forms.

[0108] Specific substitutions of individual amino acids through introduction of site-directed mutations are well-known and may be made according to methodologies with which those having ordinary skill in the art will be familiar.

The effects on catalytic activity of the resulting mutant PTP may be determined empirically by testing the resulting modified protein for the preservation of the Km and reduction of Kcat to less than 1 per minute as provided herein and as previously disclosed (e.g., WO98/04712; Flint et al., 1997 *Proc. Nat. Acad. Sci. USA* 94:1680). In addition, the effect on phosphorylatation of one or more tyrosine residues of the resulting mutant PTP molecule can also be determined empirically merely by testing such a mutant for the presence of phosphotyrosine, as also provided herein, for example, following exposure of the mutant to conditions in vitro or in vivo where it may act as a phosphate acceptor for a protein tyrosine kinase.

[0109] In particular, portions of two PTP polypeptide sequences are regarded as "corresponding" amino acid sequences, regions, fragments or the like, based on a convention of numbering one PTP sequence according to amino acid position number, and then aligning the sequence to be compared in a manner that maximizes the number of amino acids that match or that are conserved residues, for example, that remain polar (e.g., D, E, K, R, H, S, T, N, Q), hydrophobic (e.g., A, P, V, L, I, M, F, W, Y) or neutral (e.g., C, G) residues at each position. Similarly, a DNA sequence encoding a candidate PTP that is to be mutated as provided herein, or a portion, region, fragment or the like, may correspond to a known wildtype PTP-encoding DNA sequence according to a convention for numbering nucleic acid sequence positions in the known wildtype PTP DNA sequence, whereby the candidate PTP DNA sequence is aligned with the known PTP DNA such that at least 70%, preferably at least 80% and more preferably at least 90% of the nucleotides in a given sequence of at least 20 consecutive nucleotides of a sequence are identical. In certain preferred embodiments, a candidate PTP DNA sequence is greater than 95% identical to a corresponding known PTP DNA sequence. In certain particularly preferred embodiments, a portion, region or fragment of a candidate PTP DNA sequence is identical to a corresponding known PTP DNA sequence. As is well known in the art, an individual whose DNA contains no irregularities (e.g., a common or prevalent form) in a particular gene responsible for a given trait may be said to possess a wildtype genetic complement (genotype) for that gene, while the presence of irregularities known as mutations in the DNA for the gene, for example, substitutions, insertions or deletions of one or more nucleotides, indicates a mutated or mutant genotype. The invention need not be so limited, however, and contemplates other embodiments wherein two or more non-PTP polypeptides of interest (e.g., as siRNA targets), such as MAP kinase kinases or chemotherapeutic target polypeptides, are structurally related and have portions of polypeptide sequences that may be regarded as "corresponding" amino acid sequences, regions, fragments or the like, according to the alignment and identity criteria discussed above.

[0110] Modification of DNA may be performed by a variety of methods, including site-specific or site-directed mutagenesis of DNA encoding the polypeptide of interest (e.g., a siRNA target polypeptide) and the use of DNA amplification methods using primers to introduce and amplify alterations in the DNA template, such as PCR splicing by overlap extension (SOE). Site-directed mutagenesis is typically effected using a phage vector that has single- and double-stranded forms, such as M13 phage vectors, which are well-known and commercially available. Other suitable vectors that contain a single-stranded phage origin of replication may be used (see, e.g., Veira et al., *Meth. Enzymol.* 15:3, 1987). In general, site-directed mutagenesis is performed by preparing a single-stranded vector that encodes the protein of interest (e.g., a member of the PTP family, a MAP kinase kinase, or a chemotherapeutic target polypeptide). An oligonucleotide primer that contains the desired mutation within a region of homology to the DNA in the single-stranded vector is annealed to the vector followed by addition of a DNA polymerase, such as *E. coli* DNA polymerase I (Klenow fragment), which uses the double stranded region as a primer to produce a heteroduplex in which one strand encodes the altered sequence and the other the original sequence. Additional disclosure relating to site-directed mutagenesis may be found, for example, in Kunkel et al. (*Methods in Enzymol.* 154:367, 1987) and in U.S. Pat. Nos. 4,518,584 and 4,737,462. The heteroduplex is introduced into appropriate bacterial cells, and clones that include the desired mutation are selected. The resulting altered DNA molecules may be expressed recombinantly in appropriate host cells to produce the modified protein.

[0111] SiRNAs of the invention may be fused to other nucleotide molecules, or to polypeptides, in order to direct their delivery or to accomplish other functions. Thus, for example, fusion proteins comprising a siRNA oligonucleotide that is capable of specifically interfering with expression of a target polypeptide may comprise affinity tag polypeptide sequences, which refers to polypeptides or peptides that facilitate detection and isolation of the such polypeptide via a specific affinity interaction with a ligand. The ligand may be any molecule, receptor, counterreceptor, antibody or the like with which the affinity tag may interact through a specific binding interaction as provided herein. Such peptides include, for example, poly-His or "FLAG®" or the like, e.g., the antigenic identification peptides described in U.S. Pat. No. 5,011,912 and in Hopp et al., (1988 *Bio/Technology* 6:1204), or the XPRESS™ epitope tag (Invitrogen, Carlsbad, Calif.). The affinity sequence may be a hexa-histidine tag as supplied, for example, by a pBAD/His (Invitrogen) or a pQE-9 vector to provide for purification of the mature polypeptide fused to the marker in the case of a bacterial host, or, for example, the affinity sequence may be a hemagglutinin (HA) tag when a mammalian host, e.g., COS-7 cells, is used. The HA tag corresponds to an antibody defined epitope derived from the influenza hemagglutinin protein (Wilson et al., 1984 *Cell* 37:767).

[0112] The present invention also relates to vectors and to constructs that include or encode siRNA polynucleotides of the present invention, and in particular to "recombinant nucleic acid constructs" that include any nucleic acids that may be transcribed to yield target polynucleotide-specific siRNA polynucleotides (i.e., siRNA specific for a polynucleotide that encodes a target polypeptide, such as a mRNA) according to the invention as provided above; to host cells which are genetically engineered with vectors and/or constructs of the invention and to the production of siRNA polynucleotides, polypeptides, and/or fusion proteins of the invention, or fragments or variants thereof, by recombinant techniques. SiRNA sequences disclosed herein as RNA polynucleotides may be engineered to produce corresponding DNA sequences using well established methodologies such as those described herein. Thus, for example, a DNA polynucleotide may be generated from any siRNA sequence

described herein (including in the Sequence Listing), such that the present siRNA sequences will be recognized as also providing corresponding DNA polynucleotides (and their complements). These DNA polynucleotides are therefore encompassed within the contemplated invention, for example, to be incorporated into the subject invention recombinant nucleic acid constructs from which siRNA may be transcribed.

[0113] According to the present invention, a vector may comprise a recombinant nucleic acid construct containing one or more promoters for transcription of an RNA molecule, for example, the human U6 snRNA promoter (see, e.g., Miyagishi et al, *Nat. Biotechnol.* 20:497-500 (2002); Lee et al., *Nat. Biotechnol.* 20:500-505 (2002); Paul et al., *Nat. Biotechnol.* 20:505-508 (2002); Grabarek et al., *BioTechniques* 34:73544 (2003); see also Sui et al., *Proc. Natl. Acad. Sci. USA* 99:5515-20 (2002)). Each strand of a siRNA polynucleotide may be transcribed separately each under the direction of a separate promoter and then may hybridize within the cell to form the siRNA polynucleotide duplex. Each strand may also be transcribed from separate vectors (see Lee et al., supra). Alternatively, the sense and antisense sequences specific for a PTP1B sequence may be transcribed under the control of a single promoter such that the siRNA polynucleotide forms a hairpin molecule (Paul et al., supra). In such an instance, the complementary strands of the siRNA specific sequences are separated by a spacer that comprises at least four nucleotides, but may comprise at least 5, 6, 7, 8, 9, 10, 11, 12, 14, 16, 94 18 nucleotides or more nucleotides as described herein. In addition, siRNAs transcribed under the control of a U6 promoter that form a hairpin may have a stretch of about four uridines at the 3' end that act as the transcription termination signal (Miyagishi et al., supra; Paul et al., supra). By way of illustration, if the target sequence is 19 nucleotides, the siRNA hairpin polynucleotide (beginning at the 5' end) has a 19-nucleotide sense sequence followed by a spacer (which as two uridine nucleotides adjacent to the 3' end of the 19-nucleotide sense sequence), and the spacer is linked to a 19 nucleotide antisense sequence followed by a 4-uridine terminator sequence, which results in an overhang. SiRNA polynucleotides with such overhangs effectively interfere with expression of the target polypeptide (see id.). A recombinant construct may also be prepared using another RNA polymerase III promoter, the H1 RNA promoter, that may be operatively linked to siRNA polynucleotide specific sequences, which may be used for transcription of hairpin structures comprising the siRNA specific sequences or separate transcription of each strand of a siRNA duplex polynucleotide (see, e.g., Brummelkamp et al., Science 296:550-53 (2002); Paddison et al., supra). DNA vectors useful for insertion of sequences for transcription of an siRNA polynucleotide include pSUPER vector (see, e.g., Brummelkamp et al., supra); pAV vectors derived from pCWRSVN (see, e.g., Paul et al., supra); and pIND (see, e.g., Lee et al., supra), or the like.

[0114] PTP polypeptides and other target polypeptides of interest can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters, providing ready systems for evaluation of siRNA polynucleotides that are capable of interfering with polypeptide expression as provided herein. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described, for example, by Sambrook, et al., *Molecular Cloning: A Laboratory Manual*, Third Edition, Cold Spring Harbor, N.Y., (2001).

[0115] Generally, recombinant expression vectors for use in the preparation of recombinant nucleic acid constructs or vectors of the invention will include origins of replication and selectable markers permitting transformation of the host cell, e.g., the ampicillin resistance gene of *E. coli* and *S. cerevisiae* TRP1 gene, and a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence (e.g., a siRNA polynucleotide sequence). Such promoters can be derived from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), α-factor, acid phosphatase, or heat shock proteins, among others. For PTP polypeptide expression (including PTP fusion proteins and substrate trapping mutant PTPs), and for other expression of other polypeptides of interest, the heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences. Optionally, the heterologous sequence can encode a fusion protein including an N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product.

[0116] Useful expression constructs for bacterial use are constructed by inserting into an expression vector a structural DNA sequence encoding a desired siRNA polynucleotide, together with suitable transcription initiation and termination signals in operable linkage, for example, with a functional promoter. The construct may comprise one or more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector construct and, if desirable, to provide amplification within the host. Suitable prokaryotic hosts for transformation include *E. coli, Bacillus subtilis, Salmonella typhimurium* and various species within the genera Pseudomonas, Streptomyces, and Staphylococcus, although others may also be employed as a matter of choice. Any other plasmid or vector may be used as long as they are replicable and viable in the host.

[0117] As a representative but nonlimiting example, useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and GEM1 (Promega Biotec, Madison, Wis., USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed.

[0118] Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter, if it is a regulated promoter as provided herein, is induced by appropriate means (e.g., temperature shift or chemical induction) and cells are cultured for an additional period. Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification. Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents; such methods are well know to those skilled in the art.

[0119] Thus, for example, the nucleic acids of the invention as described herein (e.g., DNA sequences from which

siRNA may be transcribed) herein may be included in any one of a variety of expression vector constructs as a recombinant nucleic acid construct for expressing a target polynucleotide-specific siRNA polynucleotide. Such vectors and constructs include chromosomal, nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40; bacterial plasmids; phage DNA; baculovirus; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA, such as vaccinia, adenovirus, fowl pox virus, and pseudorabies. However, any other vector may be used for preparation of a recombinant nucleic acid construct as long as it is replicable and viable in the host.

[0120] The appropriate DNA sequence(s) may be inserted into the vector by a variety of procedures. In general, the DNA sequence is inserted into an appropriate restriction endonuclease site(s) by procedures known in the art. Standard techniques for cloning, DNA isolation, amplification and purification, for enzymatic reactions involving DNA ligase, DNA polymerase, restriction endonucleases and the like, and various separation techniques are those known and commonly employed by those skilled in the art. A number of standard techniques are described, for example, in Ausubel et al. (1993 *Current Protocols in Molecular Biology*, Greene Publ. Assoc. Inc. & John Wiley & Sons, Inc., Boston, Mass.); Sambrook et al. (2001 *Molecular Cloning*, Third Ed., Cold Spring Harbor Laboratory, Plainview, N.Y.); Maniatis et al. (1982 Molecular Cloning, Cold Spring Harbor Laboratory, Plainview, N.Y.); and elsewhere.

[0121] The DNA sequence in the expression vector is operatively linked to at least one appropriate expression control sequences (e.g., a promoter or a regulated promoter) to direct mRNA synthesis. Representative examples of such expression control sequences include LTR or SV40 promoter, the *E. coli* lac or trp, the phage lambda PL promoter and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses. Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vectors are pKK232-8 and pCM7. Particular named bacterial promoters include lac, lacZ, T3, T7, gpt, lambda P_R, P_L and trp. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art, and preparation of certain particularly preferred recombinant expression constructs comprising at least one promoter or regulated promoter operably linked to a nucleic acid encoding a polypeptide (e.g., PTP, MAP kinase kinase, or chemotherapeutic target polypeptide) is described herein.

[0122] As noted above, in certain embodiments the vector may be a viral vector such as a retroviral vector. For example, retroviruses from which the retroviral plasmid vectors may be derived include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, retroviruses such as Rous Sarcoma Virus, Harvey Sarcoma virus, avian leukosis virus, gibbon ape leukemia virus, human immunodeficiency virus, adenovirus, Myeloproliferative Sarcoma Virus, and mammary tumor virus.

[0123] The viral vector includes one or more promoters. Suitable promoters which may be employed include, but are not limited to, the retroviral LTR; the SV40 promoter; and the human cytomegalovirus (CMV) promoter described in Miller, et al., *Biotechniques* 7:980-990 (1989), or any other promoter (e.g., cellular promoters such as eukaryotic cellular promoters including, but not limited to, the histone, pol III, and β-actin promoters). Other viral promoters which may be employed include, but are not limited to, adenovirus promoters, thymidine kinase (TK) promoters, and B19 parvovirus promoters. The selection of a suitable promoter will be apparent to those skilled in the art from the teachings contained herein, and may be from among either regulated promoters or promoters as described above.

[0124] The retroviral plasmid vector is employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be transfected include, but are not limited to, the PE501, PA317, ψ-2, ψ-AM, PA12, T19-14X, VT-19-17-H2, ψCRE, ψCRIP, GP+E-86, GP+envAm12, and DAN cell lines as described in Miller, *Human Gene Therapy,* 1:5-14 (1990), which is incorporated herein by reference in its entirety. The vector may transduce the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and calcium phosphate precipitation. In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host.

[0125] The producer cell line generates infectious retroviral vector particles that include the nucleic acid sequence(s) encoding the PTP polypeptides or other polypeptide of interest and fusion proteins thereof. Such retroviral vector particles then may be employed, to transduce eukaryotic cells, either in vitro or in vivo. The transduced eukaryotic cells will express the nucleic acid sequence(s) encoding the siRNA polynucleotide that is capable of specifically interfering with expression of a polypeptide or fusion protein. Eukaryotic cells which may be transduced include, but are not limited to, embryonic stem cells, embryonic carcinoma cells, as well as hematopoietic stem cells, hepatocytes, fibroblasts, myoblasts, keratinocytes, endothelial cells, bronchial epithelial cells and various other culture-adapted cell lines.

[0126] In another aspect, the present invention relates to host cells containing the above described recombinant PTP expression constructs and to host cells containing the above described recombinant expression constructs comprising a (non-PTP) polypeptide of interest as described herein. Host cells are genetically engineered (transduced, transformed or transfected) with the vectors and/or expression constructs of this invention that may be, for example, a cloning vector, a shuttle vector, or an expression construct. The vector or construct may be, for example, in the form of a plasmid, a viral particle, a phage, etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying particular genes such as genes encoding siRNA polynucleotides or fusion proteins thereof. The culture conditions for particular host cells selected for expression, such as temperature, pH and the like, will be readily apparent to the ordinarily skilled artisan.

[0127] The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Representative examples of appropriate host

cells according to the present invention include, but need not be limited to, bacterial cells, such as *E. coli*, Streptomyces, *Salmonella typhimurium*; fungal cells, such as yeast; insect cells, such as Drosophila S2 and Spodoptera S19; animal cells, such as CHO, COS or 293 cells; adenoviruses; plant cells, or any suitable cell already adapted to in vitro propagation or so established de novo. The selection of an appropriate host is deemed to be within the scope of those skilled in the art from the teachings herein.

[0128] Various mammalian cell culture systems can also be employed to produce siRNA polynucleotides from recombinant nucleic acid constructs of the present invention. The invention is therefore directed in part to a method of producing a siRNA polynucleotide, by culturing a host cell comprising a recombinant nucleic acid construct that comprises at least one promoter operably linked to a nucleic acid sequence encoding a siRNA polynucleotide specific for a desired target polypeptide. In certain embodiments, the promoter may be a regulated promoter as provided herein, for example a tetracylcine-repressible promoter. In certain embodiments the recombinant expression construct is a recombinant viral expression construct as provided herein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman, *Cell* 23:175 (1981), and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa, HEK, and BHK cell lines. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences, for example as described herein regarding the preparation of recombinant siRNA polynucleotide constructs. DNA sequences derived from the SV40 splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements. Introduction of the construct into the host cell can be effected by a variety of methods with which those skilled in the art will be familiar, including but not limited to, for example, liposomes including cationic liposomes, calcium phosphate transfection, DEAF-Dextran mediated transfection, or electroporation (Davis et al., 1986 *Basic Methods in Molecular Biology*), or other suitable technique.

[0129] The expressed recombinant siRNA polynucleotides may be useful in intact host cells; in intact organelles such as cell membranes, intracellular vesicles or other cellular organelles; or in disrupted cell preparations including but not limited to cell homogenates or lysates, microsomes, uni- and multilamellar membrane vesicles or other preparations. Alternatively, expressed recombinant siRNA polynucleotides can be recovered and purified from recombinant cell cultures by methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

[0130] Samples

[0131] According to the present invention, a method is provided for interfering with expression of a desired target polypeptide as provided herein, comprising contacting a siRNA polynucleotide with a cell that is capable of expressing the target polypeptide, typically in a biological sample or in a subject or biological source. A "sample" as used herein refers to a biological sample containing at least one protein tyrosine phosphatase or a MAP kinase kinase or a chemotherapeutic target polypeptide, and may be provided by obtaining a blood sample, biopsy specimen, tissue explant, organ culture or any other tissue or cell preparation from a subject or a biological source. A sample may further refer to a tissue or cell preparation in which the morphological integrity or physical state has been disrupted, for example, by dissection, dissociation, solubilization, fractionation, homogenization, biochemical or chemical extraction, pulverization, lyophilization, sonication or any other means for processing a sample derived from a subject or biological source. In certain preferred embodiments, the sample is a cell that comprises at least one PTP and/or at least one MAP kinase, and/or at least one MAP kinase kinase, and in certain particularly preferred embodiments the cell comprises an inducible biological signaling pathway, at least one component of which is a specific target polypeptidee. In particularly preferred embodiments the cell is a mammalian cell, for example, Rat-1 fibroblasts, COS cells, CHO cells, HEK-293 cells, HepG2, HII4E-C3, L6, and 3T3-L1, or other well known model cell lines, which are available from the American Type Culture Collection (ATCC, Manassas, Va.). In other preferred embodiments, the cell line is derived from PTP-1B knockout animals and which may be transfected with human insulin receptor (HIR), for example, 1BKO mouse embryo fibroblasts.

[0132] In certain other preferred embodiments the sample is a cell that comprises a chemotherapeutic target polypeptide, which includes, for example, a cell line that is derived from a tumor cell. The cell line may be a primary tumor cell line, that is, a cell line prepared directly from a tumor sample removed from a human or a non-human animal. Alternatively, the cell line may be one of several established tumor cell lines known in the art, including but not limited to MCF7, T47D, SW620, HS578T, MDA-MB-435, MDA MB 231, HCT-116, HT-29, HeLa, Raji, Ramos, and the like (see ATCC collection).

[0133] The subject or biological source may be a human or non-human animal, a primary cell culture or culture adapted cell line including but not limited to genetically engineered cell lines that may contain chromosomally integrated or episomal recombinant nucleic acid sequences, immortalized or immortalizable cell lines, somatic cell hybrid cell lines, differentiated or differentiatable cell lines, transformed cell lines and the like. Optionally, in certain situations it may be desirable to treat cells in a biological sample with hydrogen peroxide and/or with another agent that directly or indirectly promotes reactive oxygen species (ROS) generation, including biological stimuli as described herein; in certain other situations it may be desirable to treat cells in a biological sample with a ROS scavenger, such as N-acetyl cysteine (NAC) or superoxide dismutase (SOD) or other ROS scavengers known in the art; in other situations cellular glutathione (GSH) may be depleted by treating cells with L-buthionine-SR-sulfoximine (Bso); and in other circumstances cells may be treated with pervanadate to enrich the sample in tyrosine phosphorylated proteins. Other means may also be employed to effect an increase in the population of tyrosine phosphorylated proteins present in the sample,

including the use of a subject or biological source that is a cell line that has been transfected with at least one gene encoding a protein tyrosine kinase.

[0134] Additionally or alternatively, a biological signaling pathway may be induced in subject or biological source cells by contacting such cells with an appropriate stimulus, which may vary depending upon the signaling pathway under investigation, whether known or unknown. For example, a signaling pathway that, when induced, results in protein tyrosine phosphorylation and/or protein tyrosine dephosphorylation may be stimulated in subject or biological source cells using any one or more of a variety of well known methods and compositions known in the art to stimulate protein tyrosine kinase (PTK) and/or PTP activity. These stimuli may include, without limitation, exposure of cells to cytokines, growth factors, hormones, peptides, small molecule mediators, cell stressors (e.g., ultraviolet light; temperature shifts; osmotic shock; ROS or a source thereof, such as hydrogen peroxide, superoxide, ozone, etc. or any agent that induces or promotes ROS production (see, e.g., Halliwell and Gutteridge, *Free Radicals in Biology and Medicine (*3rd Ed.) 1999 Oxford University Press, Oxford, UK); heavy metals; alcohol) or other agents that induce PTK-mediated protein tyrosine phosphorylation and/or PTP-mediated phosphoprotein tyrosine dephosphorylation. Such agents may include, for example, interleukins (e.g., IL-1, IL-3), interferons (e.g., IFN-γ), human growth hormone, insulin, epidermal growth factor (EGF), platelet derived growth factor (PDGF), granulocyte colony stimulating factor (G-CSF), granulocyte-megakaryocyte colony stimulating factor (GM-CSF), transforming growth factor (e.g., TGF-β1), tumor necrosis factor (e.g., TNF-α) and fibroblast growth factor (FGF; e.g., basic FGF (bFGF)), any agent or combination of agents capable of triggering T lymphocyte activation via the T cell receptor for antigen (TCR; TCR-inducing agents may include superantigens, specifically recognized antigens and/or MHC-derived peptides, MHC peptide tetramers (e.g., Altman et al., 1996 *Science* 274:94-96); TCR-specific antibodies or fragments or derivatives thereof), lectins (e.g., PHA, PWM, ConA, etc.), mitogens, G-protein coupled receptor agonists such as angiotensin-2, thrombin, thyrotropin, parathyroid hormone, lysophosphatidic acid (LPA), sphingosine-1-phosphate, serotonin, endothelin, acetylcholine, platelet activating factor (PAF) or bradykinin, as well as other agents with which those having ordinary skill in the art will be familiar (see, e.g., Rhee et al., [online] Oct. 10, 2000 *Science's stke*, Internet:URL<www.stke.org/cgl/content/full/OC-_sigtrans;2000/53/pel>), and references cited therein).

[0135] As noted above, regulated tyrosine phosphorylation contributes to specific pathways for biological signal transduction, including those associated with cell division, cell survival, apoptosis, proliferation and differentiation, and "inducible signaling pathways" in the context of the present invention include transient or stable associations or interactions among molecular components involved in the control of these and similar processes in cells. Depending on the particular pathway of interest, an appropriate parameter for determining induction of such pathway may be selected. For example, for signaling pathways associated with cell proliferation, a variety of well known methodologies are available for quantifying proliferation, including, for example, incorporation of tritiated thymidine into cellular DNA, monitoring of detectable (e.g., fluorimetric or colorimetric) indicators of cellular respiratory activity, (e.g., MTT assay) or cell counting, or the like. Similarly, in the cell biology arts there are known multiple techniques for assessing cell survival (e.g., vital dyes, metabolic indicators, etc.) and for determining apoptosis (e.g., annexin V binding, DNA fragmentation assays, caspase activation, PARP cleavage, etc.). Other signaling pathways will be associated with particular cellular phenotypes, for example specific induction of gene expression (e.g., detectable as transcription or translation products, or by bioassays of such products, or as nuclear localization of cytoplasmic factors), altered (e.g., statistically significant increases or decreases) levels of intracellular mediators (e.g., activated kinases or phosphatases, altered levels of cyclic nucleotides or of physiologically active ionic species, etc.), altered cell cycle profiles, or altered cellular morphology, and the like, such that cellular responsiveness to a particular stimulus as provided herein can be readily identified to determine whether a particular cell comprises an inducible signaling pathway.

[0136] In preferred embodiments where a siRNA of the invention is being used to interfere with expression of a target polypeptide that is a PTP or that is a component of a biological signaling pathway that comprises a PTP, a PTP substrate may be any naturally or non-naturally occurring phosphorylated peptide, polypeptide or protein that can specifically bind to and/or be dephosphorylated by a PTP (including dual specificity phosphatases) as provided herein, or any other phosphorylated molecule that can be a substrate of a PTP family member as provided herein. Non-limiting examples of known PTP substrates include the proteins VCP (see, e.g., Zhang et al., 1999 *J. Biol. Chem.* 274:17806, and references cited therein), p130^{cas}, EGF receptor, p210 bcr:abl, MAP kinase, Shc (Tiganis et al., 1998 *Mol Cell. Biol.* 18:1622-1634), insulin receptor, lck (lymphocyte specific protein tyrosine kinase, Marth et al., 1985 *Cell* 43:393), T cell receptor zeta chain, and phosphatidylinositol 3,4,5-triphosphate (Maehama et al., 1998 *J. Biol. Chem.* 273:13375).

[0137] Identification and selection of PTP substrates as provided herein, for use in the present invention, may be performed according to procedures with which those having ordinary skill in the art will be familiar, or may, for example, be conducted according to the disclosures of WO 00/75339, U.S. application Ser. No. 09/334,575, or U.S. application Ser. No. 10/366,547, and references cited therein. The phosphorylated protein/PTP complex may be isolated, for example, by conventional isolation techniques as described in U.S. Pat. No. 5,352,660, including salting out, chromatography, electrophoresis, gel filtration, fractionation, absorption, polyacrylamide gel electrophoresis, agglutination, combinations thereof or other strategies. PTP substrates that are known may also be prepared according to well known procedures that employ principles of molecular biology and/or peptide synthesis (e.g., Ausubel et al., *Current Protocols in Molecular Biology*, Greene Publ. Assoc. Inc. & John Wiley & Sons, Inc., Boston, Mass. (1993); Sambrook et al., *Molecular Cloning*, Third Ed., Cold Spring Harbor Laboratory, Plainview, N.Y. (2001); Fox, *Molec. Biotechnol.* 3:249 (1995); Maeji et al., *Pept. Res.* 8:33 (1995)).

[0138] The PTP substrate peptides of the present invention may therefore be derived from PTP substrate proteins, polypeptides and peptides as provided herein having amino acid sequences that are identical or similar to tyrosine

phosphorylated PTP substrate sequences known in the art. For example by way of illustration and not limitation, peptide sequences derived from the known PTP substrate proteins referred to above are contemplated for use according to the instant invention, as are peptides having at least 70% similarity (preferably 70% identity), more preferably 80% similarity (more preferably 80% identity), more preferably 90% similarity (more preferably 90% identity) and still more preferably 95% similarity (still more preferably 95% identity) to the polypeptides described in references cited herein and in the Examples and to portions of such polypeptides as disclosed herein. As known in the art "similarity" between two polypeptides is determined by comparing the amino acid sequence and conserved amino acid substitutes thereto of the polypeptide to the sequence of a second polypeptide (e.g., using GENEWORKS, Align or the BLAST algorithm, or another algorithm, as described above).

[0139] In certain preferred embodiments of the present invention, the siRNA polynucleotide and/or the PTP substrate is detectably labeled, and in particularly preferred embodiments the siRNA polynucleotide and/or PTP substrate is capable of generating a radioactive or a fluorescent signal. The siRNA polynucleotide and/or PTP substrate can be detectably labeled by covalently or non-covalently attaching a suitable reporter molecule or moiety, for example a radionuclide such as ^{32}P (e.g., Pestka et al., 1999 *Protein Expr. Purif.* 17:203-14), a radiohalogen such as iodine [^{125}I or ^{131}I] (e.g., Wilbur, 1992 *Bioconjug. Chem.* 3:433-70), or tritium [^{3}H]; an enzyme; or any of various luminescent (e.g., chemiluminescent) or fluorescent materials (e.g., a fluorophore) selected according to the particular fluorescence detection technique to be employed, as known in the art and based upon the present disclosure. Fluorescent reporter moieties and methods for labeling siRNA polynucleotides and/or PTP substrates as provided herein can be found, for example in Haugland (1996 *Handbook of Fluorescent Probes and Research Chemicals—Sixth Ed.*, Molecular Probes, Eugene, Oreg.; 1999 *Handbook of Fluorescent Probes and Research Chemicals—Seventh Ed.*, Molecular Probes, Eugene, Oreg., Internet: http://www.probes.com/lit/) and in references cited therein. Particularly preferred for use as such a fluorophore in the subject invention methods are fluorescein, rhodamine, Texas Red, AlexaFluor-594, AlexaFluor-488, Oregon Green, BODIPY-FL, umbelliferone, dichlorotriazinylamine fluorescein, dansyl chloride, phycoerythrin or Cy-5. Examples of suitable enzymes include, but are not limited to, horseradish peroxidase, biotin, alkaline phosphatase, β-galactosidase and acetylcholinesterase. Appropriate luminescent materials include luminol, and suitable radioactive materials include radioactive phosphorus [^{32}P]. In certain other preferred embodiments of the present invention, a detectably labeled siRNA polynucleotide comprises a magnetic particle, for example a paramagnetic or a diamagnetic particle or other magnetic particle or the like (preferably a microparticle) known to the art and suitable for the intended use. Without wishing to be limited by theory, according to certain such embodiments there is provided a method for selecting a cell that has bound, adsorbed, absorbed, internalized or otherwise become associated with a siRNA polynucleotide that comprises a magnetic particle. For example, selective isolation of a population or subpopulation of cells containing one or more PTP-specific siRNA polynucleotide-magnetic particle conjugates may offer certain advantages in the further characterization or regulation of PTP signaling pathways.

[0140] In certain embodiments of the present invention, particular PTP-specific siRNA polynucleotides of interest may be identified by contacting a candidate siRNA polynucleotide with a sample comprising a cell that comprises a target polypeptide-encoding gene and that is capable of target polypeptide gene transcription or expression (e.g., translation), under conditions and for a time sufficient to detect such gene transcription or expression, and comparing target transcription levels, polypeptide expression and/or functional expression (e.g., PTP catalytic activity) in the absence and presence of the candidate siRNA polynucleotide. Preferably target transcription or expression is decreased in the presence of the siRNA polynucleotide, which in the case of targets that are PTPs provides an alternative to PTP active site directed approaches to modulating PTP activity. (The invention need not be so limited, however, and contemplates other embodiments wherein transcription and/or expression levels of a signal transduction component other than that which is specifically targeted by the siRNA may be increased in the presence of a certain target-specific siRNA polynucleotide. By way of non-limiting theory, such an increase may result from a cellular compensatory mechanism that is induced as a result of the siRNA.)

[0141] Activity of a siRNA target polypeptide of interest may also be measured in whole cells transfected with a reporter gene whose expression is dependent upon the activation of an appropriate substrate. For example, appropriate cells (i.e., cells that express the target polypeptide and that have also been transfected with a target-specific siRNA polynucleotide that is either known or suspected of being capable of interfering with target polypeptide expression) may be transfected with a substrate-dependent promoter linked to a reporter gene. In such a system, expression of the reporter gene (which may be readily detected using methods well known to those of ordinary skill in the art) depends upon activation of the substrate via its interaction with the target polypeptide. For example, dephosphorylation of substrate may be detected based on a decrease in reporter activity in situations where the target polypeptide regulates substrate phosphorylation.

[0142] Within other aspects, the present invention provides animal models in which an animal, by virtue of introduction of an appropriate target polypeptide-specific siRNA polynucleotide, for example, as a transgene, does not express (or expresses a significantly reduced amount of) a functional PTP. Such animals may be generated, for example, using standard homologous recombination strategies, or alternatively, for instance, by oocyte microinjection with a plasmid comprising the siRNA-encoding sequence that is regulated by a suitable promoter (e.g., ubiquitous or tissue-specific) followed by implantation in a surrogate mother. Animal models generated in this manner may be used to study activities of PTP signaling pathway components and modulating agents in vivo.

[0143] Therapeutic Methods

[0144] One or more siRNA polynucleotides capable of interfering with target polypeptide expression and identified according to the above-described methods may also be used to modulate (e.g., inhibit or potentiate) target polypeptide

activity in a patient. As used herein, a "patient" may be any mammal, including a human, and may be afflicted with a condition associated with undesired target polypeptide activity or may be free of detectable disease. Accordingly, the treatment may be of an existing disease or may be prophylactic. Conditions associated with signal transduction and/or with inappropriate activity of specific siRNA target polypeptides described herein include obesity, impaired glucose tolerance and diabetes and cancer, disorders associated with cell proliferation, including cancer, graft-versus-host disease (GVHD), autoimmune diseases, allergy or other conditions in which immunosuppression may be involved, metabolic diseases, abnormal cell growth or proliferation and cell cycle abnormalities.

[0145] For administration to a patient, one or more specific siRNA polynucleotides, either alone, with or without chemical modification or removal of ribose, or comprised in an appropriate vector as described herein (e.g., including a vector which comprises a DNA sequence from which a specific siRNA can be transcribed) are generally formulated as a pharmaceutical composition. A pharmaceutical composition may be a sterile aqueous or non-aqueous solution, suspension or emulsion, which additionally comprises a physiologically acceptable carrier (i.e., a non-toxic material that does not interfere with the activity of the active ingredient). Such compositions may be in the form of a solid, liquid or gas (aerosol). Alternatively, compositions of the present invention may be formulated as a lyophilizate or compounds may be encapsulated within liposomes using well known technology. Pharmaceutical compositions within the scope of the present invention may also contain other components, which may be biologically active or inactive. Such components include, but are not limited to, buffers (e.g., neutral buffered saline or phosphate buffered saline), carbohydrates (e.g., glucose, mannose, sucrose or dextrans), mannitol, proteins, polypeptides or amino acids such as glycine, antioxidants, chelating agents such as EDTA or glutathione, stabilizers, dyes, flavoring agents, and suspending agents and/or preservatives.

[0146] Any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of the present invention. Carriers for therapeutic use are well known, and are described, for example, in *Remingtons Pharmaceutical Sciences*, Mack Publishing Co. (A. R. Gennaro ed. 1985). In general, the type of carrier is selected based on the mode of administration. Pharmaceutical compositions may be formulated for any appropriate manner of administration, including, for example, topical, oral, nasal, intrathecal, rectal, vaginal, sublingual or parenteral administration, including subcutaneous, intravenous, intramuscular, intrasternal, intracavernous, intrameatal or intraurethral injection or infusion. For parenteral administration, the carrier preferably comprises water, saline, alcohol, a fat, a wax or a buffer. For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, kaolin, glycerin, starch dextrins, sodium alginate, carboxymethylcellulose, ethyl cellulose, glucose, sucrose and/or magnesium carbonate, may be employed.

[0147] A pharmaceutical composition (e.g., for oral administration or delivery by injection) may be in the form of a liquid (e.g., an elixir, syrup, solution, emulsion or suspension). A liquid pharmaceutical composition may include, for example, one or more of the following: sterile diluents such as water for injection, saline solution, preferably physiological saline, Ringer's solution, isotonic sodium chloride, fixed oils such as synthetic mono or diglycerides which may serve as the solvent or suspending medium, polyethylene glycols, glycerin, propylene glycol or other solvents; antibacterial agents such as benzyl alcohol or methyl paraben; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. A parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic. The use of physiological saline is preferred, and an injectable pharmaceutical composition is preferably sterile.

[0148] The compositions described herein may be formulated for sustained release (i.e., a formulation such as a capsule or sponge that effects a slow release of compound following administration). Such compositions may generally be prepared using well known technology and administered by, for example, oral, rectal or subcutaneous implantation, or by implantation at the desired target site. Sustained-release formulations may contain an agent dispersed in a carrier matrix and/or contained within a reservoir surrounded by a rate controlling membrane. Carriers for use within such formulations are biocompatible, and may also be biodegradable; preferably the formulation provides a relatively constant level of active component release. The amount of active compound contained within a sustained release formulation depends upon the site of implantation, the rate and expected duration of release and the nature of the condition to be treated or prevented.

[0149] Within a pharmaceutical composition, a therapeutic agent comprising a polypeptide-directed siRNA polynucleotide as described herein (or, e.g., a recombinant nucleic acid construct encoding a siRNA polynucleotide) may be linked to any of a variety of compounds. For example, such an agent may be linked to a targeting moiety (e.g., a monoclonal or polyclonal antibody, a protein or a liposome) that facilitates the delivery of the agent to the target site. As used herein, a "targeting moiety" may be any substance (such as a compound or cell) that, when linked to an agent enhances the transport of the agent to a target cell or tissue, thereby increasing the local concentration of the agent. Targeting moieties include antibodies or fragments thereof, receptors, ligands and other molecules that bind to cells of, or in the vicinity of, the target tissue. An antibody targeting agent may be an intact (whole) molecule, a fragment thereof, or a functional equivalent thereof. Examples of antibody fragments are $F(ab')_2$, Fab', Fab and F[v] fragments, which may be produced by conventional methods or by genetic or protein engineering. Linkage is generally covalent and may be achieved by, for example, direct condensation or other reactions, or by way of bi- or multi-functional linkers. Targeting moieties may be selected based on the cell(s) or tissue(s) toward which the agent is expected to exert a therapeutic benefit.

[0150] Pharmaceutical compositions may be administered in a manner appropriate to the disease to be treated (or prevented). An appropriate dosage and a suitable duration and frequency of administration will be determined by such

factors as the condition of the patient, the type and severity of the patient's disease, the particular form of the active ingredient and the method of administration. In general, an appropriate dosage and treatment regimen provides the agent(s) in an amount sufficient to provide therapeutic and/or prophylactic benefit (e.g., an improved clinical outcome, such as more frequent complete or partial remissions, or longer disease-free and/or overall survival, or a lessening of symptom severity). For prophylactic use, a dose should be sufficient to prevent, delay the onset of or diminish the severity of a disease associated with cell proliferation.

[0151] Optimal dosages may generally be determined using experimental models and/or clinical trials. In general, the amount of siRNA polynucleotide present in a dose, or produced in situ by DNA present in a dose (e.g., from a recombinant nucleic acid construct comprising a siRNA polynucleotide), ranges from about 0.01 μg to about 1001 g per kg of host, typically from about 0.1 μg to about 10 μg. The use of the minimum dosage that is sufficient to provide effective therapy is usually preferred. Patients may generally be monitored for therapeutic or prophylactic effectiveness using assays suitable for the condition being treated or prevented, which will be familiar to those having ordinary skill in the art. Suitable dose sizes will vary with the size of the patient, but will typically range from about 10 mL to about 500 mL for 10-60 kg animal.

[0152] The following Examples are offered by way of illustration and not by way of limitation.

EXAMPLE 1

Interference of Dual Specificity Phosphatase Expression by Small Interfering RNA

[0153] This example describes the effect on dual specificity phosphatase (DSP) expression in cells transfected with sequence-specific small interfering RNA (siRNA) polynucleotides. Interference with expression of MKP-1 and DSP-3 was examined by transfecting sequence-specific siRNAs into mammalian cells expressing the DSP polypeptide and then detecting expression by immunoblot.

[0154] The siRNA nucleotide sequences specific for each DSP were chosen by first scanning the open reading frame of the target cDNA for 21-base sequences that were flanked on the 5' end by two adenine bases (AA) and that had A+T/G+C ratios that were nearly 1:1. Twenty-one-base sequences with an A+T/G+C ratio greater than 2:1 or 1:2 were excluded. If no 21-base sequences were identified that met this criteria, the polynucleotide sequence encoding the DSP was searched for a 21-base sequence having the bases CA at the 5' end. The polynucleotide sequences examined were the sequences encoding DSP-3 polypeptide (SEQ ID NO:______) and MKP-1 (SEQ ID NO:______). For the selection of sequences for some of the siRNA polynucleotides, the sense and antisense sequences of each 21-mer that met the above criteria were then analyzed to determine if the sequence had the potential to form an internal hairpin loop or homodimer. Such an analysis can be performed using computer software programs known to those in the art. Any 21-mer that had an internal hairpin loop melting temperature of greater than 55° C. and a homodimer melting temperature of greater than 37° C. was excluded. The specificity of each 21-mer was determined by performing a BLAST search of public databases. Sequences that contained at least 16 of 21 consecutive nucleotides with 100% identity with a polynucleotide sequence other than the target sequence were not used in the experiments. In each of the Examples provided herein, each siRNA sequence represents the sense strand of the siRNA polynucleotide and its corresponding sequence identifier. "Related sequence identifiers" referred to in the Examples identify sequences in the sequence listing that contain the same nucleotides at positions 1-19 of the siRNA sequence with and without two additional nucleotides (NN) at the 3' end (which would correspond to a two-nucleotide overhang in a double stranded polynucleotide), and the reverse complement of each. Unless otherwise stated, it is to be understood that the siRNA transfected into a cell is composed of the sense strand and its complementary antisense strand, which form a duplex siRNA polynucleotide. The sequences chosen for these experiments were as follows.

[0155] DSP-3 Specific:

```
DSP3.1:
5'-cgauagugccaggccuaugtt-3'   [SEQ ID NO:___]

DSP3.2:
5'-gcaugagguccaucaguautt-3'   [SEQ ID NO:___]

DSP3.3:
5'-cgauacugccaggcccaugtt-3'   [SEQ ID NO:___]
```

[0156] MKP-1 Specific:

```
MKP.1: 5'-auccugcccuuucuguacctt-3' [SEQ ID NO:___]

MKP.2: 5'-gcagaggcaaagcaucauctt-3' [SEQ ID NO:___]
```

[0157] Sense and antisense oligonucleotides for MKP.1, MKP.2, DSP3.1, DSP3.2, and DSP3.3 were synthesized according to the standard protocol of the vendor (Dharmacon Research, Inc., Lafayette, Colo.). For some experiments described in this and other examples, the vendor gel-purified the double-stranded siRNA polynucleotide, which was then used. In the instances when the vendor did not prepare double-stranded siRNA, just before transfection, double-stranded siRNAs were prepared by annealing the sense and anti-sense oligonucleotides in annealing buffer (100 mM potassium acetate, 30 mM HEPES-KOH, pH 7.4, 2 mM magnesium acetate) for 1 minute at 90° C., followed by a 60 minute incubation at 37° C.

[0158] Recombinant nucleic acid expression vectors containing encoding sequences for the MKP-1 polypeptide and DSP-3 polypeptide were prepared according to standard molecular biology techniques. Polynucleotides comprising the MKP-1 coding sequence of SEQ ID NO:______ and comprising the DSP-3 coding sequence of SEQ ID NO:______ were cloned into recombinant expression vectors according to methods known to those skilled in the molecular biology art.

[0159] HeLa cells (ATCC, Manassas, Va.) were maintained in Dulbecco's modified Eagle's medium (DMEM, Life Technologies, Inc., Gaithersburg, Md.) plus 10% fetal bovine serum (FBS), 100 units/ml penicillin, and 100 μg/ml streptomycin. Cells were plated in 6-well tissue culture plates at a density of approximately 5×10^4 cells per well at the time of transfection.

[0160] HeLa cells were transfected with 60 pmoles of MKP.1, MKP.2, or CD45.1 (SEQ ID NO:______) siRNA. For each cell culture well, the siRNA polynucleotides were diluted into 250 μl of O_{PTI}MEM® Reduced Serum Medium (Gibco™, Life Technologies), and 15 μl Oligofectamine™ (Invitrogen Life Technologies, Carlsbad, Calif.) was diluted into 250 μl of O_{PTI}MEM®. A control solution without siRNA was also prepared. Each solution was incubated at room temperature for 5 minutes. The two solutions were mixed and then incubated for 20 minutes at room temperature to allow the liposome-nucleic acid complexes to form. FBS-containing media was removed from the HeLa cell cultures and replaced with O_{PTI}MEM®. The liposome-nucleic acid mixture then was added to the HeLa cell culture, and the transfected cells incubated at 37° C. for 22-24 hours. Media were removed from the cell cultures and replaced with DMEM containing 10% FBS. Cells were incubated at 37° C. in the media plus FBS solution for 0, 1, or 4 hours.

[0161] Expression of MKP-1 was analyzed by immunoblotting HeLa cell extracts. The cells were rinsed twice in phosphate buffered saline (PBS) (4° C.) and then lysed in 250 μl of ice-cold RIPA buffer RIPA buffer (150 mM NaCl, 10 mM $NaPO_4$, 2 mM EDTA, 1% deoxycholate, 1% Nonidet® P40, 0.1% SDS, 5 mM NaF, 14.3 mM beta-mercaptoethanol, and Complete Protease Inhibitor (Roche Applied Bioscience, Indianapolis, Ind.). The lysates were centrifuged and aliquots of supernatant (10 μl) from each transfected cell culture sample were combined with 10 μl of 2×SDS-PAGE reducing sample buffer. The samples were heated at 95° C. for five minutes, and then applied to a 14% Tris-glycine SDS-PAGE gel (NOVEX® from Invitrogen Life Technologies, Carlsbad, Calif.). After electrophoresis, the separated proteins were electrophoretically transferred from the gel onto an Immobilon-P polyvinylidene fluoride (PVDF) membrane (Millipore, Bedford, Mass.). The PVDF membrane was blocked in 5% milk in TBST (20 mM Tris pH 7.5, 150 mM NaCl, 0.05% Tween-20), incubated with an anti-MKP-1 antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, Calif.) for 2-16 hours at room temperature, washed 3×10 minutes with TBST, and then incubated with an appropriate horseradish peroxidase (HRP) conjugate IgG (1:10,000) (Amersham Biosciences, Piscataway, N.J.) for 30 minutes at room temperature. Binding was detected with the ECL chemiluminescent reagent used according to the manufacturer's instructions (Amersham Biosciences, Piscataway, N.J.) as shown in **FIG. 1** (upper). A second SDS-PAGE gel in which the HeLa cell extracts were separated was stained with Coomassie Blue (**FIG. 1**, lower).

[0162] Interference with DSP-3 polypeptide expression was analyzed in HeLa cells transfected with siRNA polynucleotides. To determine the transfection efficiency of a siRNA polynucleotide, HeLa cells cultured as described above were plated at different cell densities and then transfected with a sequence-specific siRNA. DSP3.1 siRNA (SEQ ID NO:______) was synthesized and conjugated to fluorescein isothiocyanate (FITC) according to the vendor's standard methods (Synthetic Genetics, San Diego, Calif.). HeLa cells plated at varying cell densities to achieve approximately 1×10^4 cells/well, 3×10^4 cells/well, 5×10^4 cells/well, 1×10^5 cells/well, 2×10^5 cells/well, and 4×10^5 cells/well were transfected with FITC-DSP3.1 as described above. Controls included HeLa cells exposed to Lipofectamine™ 2000 alone and to media alone. The transfected cells were harvested after 24-48 hours and analyzed by a fluorescence-activated cell sorter (FACS). Transfection was more efficient at cell densities of 5×10^4 cells/well or less.

[0163] Interference of DSP-3 expression by two different DSP-3 sequence specific siRNA polynucleotides, DSP3.1 (SEQ ID NO:______) and DSP3.2 (SEQ ID NO:______). Transfection of HeLa cells was performed as described for MKP-1. As controls, HeLa cells were transfected with non-specific MKP.1 (SEQ ID NO:______) and with transfection solution not containing the expression vector or siRNA.

[0164] Twenty-four hours after transfection, cell extracts were prepared either using RIPA buffer (see above) or 1% Triton X-100®. The extracts were analyzed by immunoblot (see above) using an anti-DSP-3 monoclonal antibody, clone 17, diluted 1:10,000 in TBST and binding was detected with HRP-conjugated anti-mouse IgG. DSP3.1 effectively decreased expression of DSP-3, whereas the level of expression in cells transfected with siRNA DSP3.2 was comparable to expression in the cells transfected with the non-specific MKP.1 siRNA. The cell extracts were also immunoblotted against an anti-PTP1B antibody, which demonstrated that protein expression of another protein expressed in the cells was not affected by the presence of siRNA polynucleotides. The data suggest that the decrease in the level of DSP-3 expression varies depending upon the particular sequence of the siRNA.

[0165] To evaluate the sensitivity of interference by specific siRNA polynucleotides, DSP3.1 siRNA (SEQ ID NO:______) was titrated in HeLa cells. HeLa cells were transfected as described above with DSP3.1 siRNA (SEQ ID NO:1) at a concentration of 1, 2, 5, 10, 20, and 100 nM. HeLa cells were also transfected at the same concentrations with non-specific siRNAs, cdc14a.1 (5'-caucgugcgaagguuccugtt-3' (SEQ ID NO:6)) and CD45.2 (5'-gccgagaacaaaguggaugtt-3' (SEQ ID NO:______)). An immunoblot of cell extracts prepared using RIPA buffer was probed with anti-DSP-3 monoclonal antibody clone 17. A second immunoblot was probed with an anti-JNK2 antibody. DSP-3 expression decreased to approximately the same level in cells transfected with 5, 10, 20, and 100 nM of the specific siRNA DSP3.1. The level of expression of DSP-3 also decreased in the presence of the lowest concentrations of siRNA DSP3.1 compared with DSP-3 expression in cells transfected with non-specific siRNAs. Expression of JNK2 was not affected.

[0166] The specificity of siRNA interference was demonstrated by co-transfecting HeLa cells with the DSP-3 expression vector and an siRNA, DSP3.3 (SEQ ID NO:______) that had two base differences from siDSP3.1. Transfection and immunoblotting were performed as described above for the titration experiment. The expression levels of DSP-3 polypeptide was effectively decreased in the presence of 1, 5, 10, 20, or 100 nM of DSP3.1 but not in cells transfected with DSP3.3. The level of expression of JNK2 was not affected.

EXAMPLE 2

Interference with Expression of Protein Tyrosine Phosphatases by Sequence-Specific Small Interfering RNA

[0167] This example describes RNA interference of transient and endogenous expression of various protein tyrosine phosphatases (PTPs).

[0168] Co-Transfection Assays to Determine Interference of PTP Expression by siRNA

[0169] DSP-11 and DSP-18

[0170] Interference of expression of FLAG®-tagged DSP-11 polypeptide and FLAG®-tagged DSP-18pr polypeptide (DSP-18) by sequence specific siRNA polynucleotides was determined. (FLAG® sequence: Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys (SEQ ID NO:______)) (Sigma Aldrich, St. Louis, Mo.). Two siRNA sequences that were specific for DSP-11 polynucleotide (SEQ ID NO:______) encoding a DSP-11 polypeptide (SEQ ID NO:______) and two siRNA sequences specific for DSP-18pr polynucleotide (DSP-18, SEQ ID NO:______) encoding a DSP-18 polypeptide (SEQ ID NO:______) were designed using the criteria described in Example 1. The following sequences were used in the experiments.

[0171] DSP-11 Specific:

```
DSP11.2:
5'-cuggcaccaugcuggccugtt-3'   [SEQ ID NO:___]

DSP11.4:
5'-agcagucuuccaguucuactt-3'   [SEQ ID NO:___]
```

[0172] DSP-18 Specific:

```
DSP18.2:
5'-cugccuugugcacugcuuutt-3'   [SEQ ID NO:___]

DSP18.4:
5'-gaguuuggcugggccaguutt-3'   [SEQ ID NO:___]
```

[0173] Vectors for expression of DSP-18 and DSP-11 were prepared as follows. Vector pCMVTag2B (Stratagene, La Jolla, Calif.) was digested with restriction endonuclease BamHI (New England Biolabs, Beverly, Mass.) for 3 hours at 37° C. The digested vector was then incubated with Klenow polymerase (New England Biolabs) for 15 minutes at 25° C. to fill in the recessed 3' termini, followed by an incubation of 30 minutes at 37° C. with calf intestinal phosphatase (New England Biolabs). The GATEWAY™ Reading Frame Cassette B (Invitrogen, Carlsbad, Calif.) was inserted into the pCMVTag2B vector by ligation with T4 DNA ligase (Invitrogen) overnight at 16° C. according to the supplier's instructions. DB3.1™ competent *E. coli* cells were transformed with the ligated vector (GWpCMVTag2), and DNA was isolated by standard molecular biology methods. DSP-11 and DSP-18 constructs were prepared by ligating a polynucleotide encoding DSP-11 (SEQ ID NO:25) and a polynucleotide encoding DSP-18 (SEQ ID NO:27) into a modified bacterial pGEX-6PKG expression vector (Amersham Biosciences), referred to as pGEX-6P1, according to standard methods known in the molecular biology art. DSP-11 and DSP18 constructs and the pENTR™ 1A entry vector (Invitrogen) were digested with EcoRI (New England Biolabs) for 3 hours at 37° C. The pENTR™ 1A clone was treated with calf intestinal phosphatase for 30 minutes at 37° C., and then DSP-11 and DSP-18 constructs were inserted into separate pENTR™ vectors by ligation overnight at 16° C. with T4 DNA ligase. Vector DNA was prepared from LIBRARY EFFICIENCY® DH5α™ cells (Invitrogen) that were transformed with each construct according to the supplier's recommendation.

[0174] FLAG® epitope-tagged DSP-11 and DSP-18 polypeptides were prepared by cloning the pENTR™ 1A-DSP-18 and substrate trapping mutant constructs into the GWpCMVTag2 vector. The pENTR™ 1A constructs containing the DSP-11 and the DSP-18 polynucleotides were linearized by digesting the constructs with Vsp I (Promega Corp., Madison, Wis.) for 2 hours at 37° C. The DNA was purified using a QIAGEN PCR Purification kit (QIAGEN, Inc., Valencia, Calif.), and 30 μl (100 ng/μl) was combined in a GATEWAY™ LR reaction with 6 μl linearized pENTR™ 1A-DSP-11, pENTR™ 1A-DSP-18, 3 μl TE buffer, 4 μl Clonase™ Enzyme, and 4 μl LR reaction buffer (Invitrogen) for 1 hour at room temperature. After addition of Proteinase K (Invitrogen) to each reaction for 10 minutes, LIBRARY EFFICIENCY® DH5α™ cells were transformed with each expression vector. For controls, FLAG®-DSP-3 and FLAG®-cdc14b were also prepared according to the above method.

[0175] 293-HEK cells, maintained in DMEM, 10% FBS at 37° C. and 5% CO_2, were co-transfected with the FLAG®-DSP-11, FLAG®-DSP-18, FLAG®-DSP-3, and FLAG®-cdc14b expression vectors and DSP11.2, DSP11.4, DSP18.2, and DSP18.4 siRNAs (20 nM) (double-stranded RNA was prepared as described in Example 1) using the Lipofectamine™ 2000 reagent (Invitrogen). After incubating the transfected cells for 22-24 hours at 37° C., cells were rinsed twice in phosphate buffered saline (PBS) (4° C.) and then lysed in 250 μl of ice-cold RIPA buffer (see Example 1). The cell debris was pelleted and aliquots of each supernatant were separated by SDS-PAGE and immunoblotted as described in Example 1. DSP-11 and DSP-18 polypeptides were detected by probing the immunoblots with an anti-FLAG® antibody (Sigma-Aldrich, St. Louis, Mo.) followed by probing with an HRP-conjugated goat anti-mouse reagent (see Example 1). Binding of the anti-FLAG® antibody was detected by chemiluminescence development (see Example 1). **FIG. 2** shows that expression of FLAG®-DSP-11 and FLAG®-DSP-18 was inhibited in the presence of sequence-specific siRNA.

[0176] DSP-13 and DSP-14

[0177] Expression constructs of DSP-13 (SEQ ID NO:______) and DSP-14 (SEQ ID NO:______) and FLAG® epitope-tagged DSP-13 and DSP-14 polypeptides (SEQ ID NO:______ and SEQ ID NO:______, respectively) were prepared essentially as described above. Four siRNA sequences specific for DSP-13 polynucleotide and four siRNA sequences specific for DSP-14 were designed according to the criteria described in Example 1 except that melting temperatures were not necessarily calculated. After performing the BLAST search to analyze the specificity of a sequence, sequences that contained at least 16 consecutive nucleotides with 100% identity with a polynucleotide sequence other than the target sequence were not used in the experiments. The siRNA polynucleotides were manufactured by Dharmacon Research Inc. The sequences of the siRNA polynucleotides are as follows.

[0178] DSP-13 Specific:

```
DSP13.1:
5'-cuugcgggaauucaaggaatt-3'   (SEQ ID NO:___)
```

-continued

```
DSP13.2:
5'-ccgaggguacgguauauctt-3'    (SEQ ID NO:___)

DSP13.3:
5'-caucaggcuggcuguaagatt-3'   (SEQ ID NO:___)

DSP13.4:
5'-cauggaucuaaaugccuugtt-3'   (SEQ ID NO:___)
```

[0179] DSP-14 Specific:

```
DSP-14.1:
5'-gugaagacaagccucaagatt-3'   (SEQ ID NO:___)

DSP-14.2:
5'-gcucuacauuggcgaugagtt-3'   (SEQ ID NO:___)

DSP-14.3:
5'-gcgacgaccacaguaagautt-3'   (SEQ ID NO:___)

DSP-14.4:
5'-ggacaugacccugguggactt-3'   (SEQ ID NO:___)
```

[0180] 293-HEK cells were co-transfected with 1-2 μg of the FLAG®-DSP-13 or FLAG®-DSP-14 expression vector and 20 nM of siRNA and expression detected by immunoblot as described above. As controls, cells co-transfected with a DSP expression vector and a non-specific siRNA and untransfected 293-HEK cells were included in the analysis.

[0181] The amount of of FLAG®-DSP-13 polypeptide expressed in 293-HEK cells co-transfected with the FLAG®-DSP-13 construct and either DSP13.3 or DSP13.4 siRNA decreased more than 95% compared with cells transfected with the DSP-13 expression constructs only. Expression of the DSP-13 polypeptide in cells co-transfected with DSP13.2 siRNA was comparable to expression in cells co-transfected with a non-specific siRNA (DSP14.1). Expression of FLAG®-DSP-14 polypeptide decreased 70% in 293-HEK cells when the cells were co-transfected with DSP14.1 siRNA and decreased 90% when the cells were co-transfected with DSP-14.3 siRNA. Expression of DSP-14 in the presence of siRNA 14.4 was only slightly lower than observed with a non-specific siRNA (DSP13.1).

[0182] DSP-3

[0183] Transient co-transfection experiments in 293-HEK cells were also performed with DSP3.1 siRNA (SEQ ID NO:1) and a DSP-3 polypeptide recombinant expression vector (prepared according to standard molecular biology techniques). Expression of DSP-3 was determined by immunoblot probed with anti-DSP-3 monoclonal antibody clone 17. The results showed that the amount of DSP-3 polypeptide expressed in the 293-HEK cells decreased 80% in the presence of sequence specific siRNA.

[0184] SHP-2

[0185] Inhibition of expression of the protein tyrosine phosphatase (PTP) SHP-2 (src homology protein-2) was also examined in the 293-HEK co-transfection assay. Four different siRNAs specific for the polynucleotide sequence (SEQ ID NO:______) encoding SHP-2 (SEQ ID NO:______) were co-transfected with a FLAG®-SHP-2 expression construct prepared according to the molecular biology methods described above. SHP-2 specific siRNAs had the following sequences.

```
SHP2.1:
5'-gauucagaacacuggugautt-3'   (SEQ ID NO:___)

SHP2.2:
5'-gaauauggcgucaugcgugtt-3'   (SEQ ID NO:___)

SHP2.3:
5'-cggucuggcaauaccacuutt-3'   (SEQ ID NO:___)

SHP2.4:
5'-ugacggcaagucuaaagugtt-3'   (SEQ ID NO:___)
```

[0186] The siRNA SHP2.1 effectively impaired expression of SHP-2 in transfected 293-HEK cells, decreasing the amount of FLAG®-SHP-2 polypeptide detected by more than 95%. In the presence of siRNA SHP2.2, FLAG®-SHP-2 polypeptide expression decreased by 85%. SHP2-4 had no specific effect on SHP-2 expression.

[0187] PRL-3 and KAP

[0188] Inhibition of expression of the human protein tyrosine phosphatases (PTP) PRL-3 and KAP were also examined in the 293-HEK co-transfection assay. Four different siRNAs specific for the polynucleotide sequence (SEQ ID NO:______) encoding PRL-3 (SEQ ID NO:______) were co-transfected with a FLAG®-PRL-3 expression construct prepared according to the molecular biology methods described above. Similarly, four different siRNAs specific for the polynucleotide sequence (SEQ ID NO:______) encoding KAP (SEQ ID NO:______) were co-transfected with a FLAG®-KAP expression construct. The siRNA sequences and the percent decrease in the level of expression of the PTP in cells transfected with the each siRNA is presented in Table 1 below, and it is noted that each 21-mer sequence below contains a dinucleotide "overhang" at the 3' end, and that the invention herein should be considered to include the 19-mer polynucleotide sequences beginning at the 5' end therein as well as the 21-mer polynucleotide shown in the Table.

TABLE 1

siRNA INTERFERENCE WITH PRL-3 AND KAP IN CO-TRANSFECTION ASSAYS

Target	siRNA Sequence (SEQ ID NO)	siRNA Name	Related SEQ ID NO:	Decrease in Expression
KAP	5'-GAGCCUAUUGAAGAUGAACTT-3'	KAP.1		>90%
KAP	5'-GAGCUGUGGUAUACAAGACTT-3'	KAP.2		>90%
KAP	5'-GAGCUUACAACCUGCCUUATT-3'	KAP.3		>90%

TABLE 1-continued

siRNA INTERFERENCE WITH PRL-3 AND KAP IN CO-TRANSFECTION ASSAYS

Target	siRNA Sequence (SEQ ID NO)	siRNA Name	Related SEQ ID NO:	Decrease in Expression
KAP	5'-UACACUGCUAUGGAGGACUTT-3'	KAP.4		<10%
PRL-3	5'-GUGACCUAUGACAAAACGCTT-3'	Prl3.1		50%
PRL-3	5'-GGCCAAGUUCUGUGAGGCCTT-3'	Prl3.2		50%
PRL-3	5'-GUACGAGGACGCCAUCCAGTT-3'	Prl3.3		50%
PRL-3	UACCGGCCCAAACAGAGGCTT	Prl3.4		<10%

[0189] PTPϵ

[0190] Inhibition of expression of human PTPϵ is examined in the 293-HEK co-transfection assay. Four different siRNAs specific for the polynucleotide sequence (SEQ ID NO:______) encoding PTPϵ (SEQ ID NO:______) are co-transfected with a FLAG®-PTPϵ expression construct prepared according to the molecular biology methods described above. The siRNA sequences that are analyzed have AA leader sequences (not included in the siRNA polynucleotide transfected into HEK cells) and the following sequences.

RPTPE.1: 5'GCAGAGGAAAGCUGUGGUCTT3' (SEQ ID NO:___)

RPTPE.2: 5'GUCUGCGACCAUCGUCAUGTT3' (SEQ ID NO:___)

RPTPE.3: 5'GCCUUACUCGAGUACUACCTT3' (SEQ ID NO:___)

RPTPE.4: 5'GGACUAUUUCAUCGCCACCTT3' (SEQ ID NO:___)

[0191] Interference by siRNA Polynucleotides of Endogenous PTP Expression

[0192] The effect of sequence specific siRNA polynucleotides on expression of protein tyrosine phosphatases endogenously expressed in cells was also determined. Inhibition of expression of SHP-2 in HeLa cells by specific siRNAs was examined. HeLa cells were transfected with 10 nM of SHP2.1 (SEQ ID NO:______); SHP2.2 (SEQ ID NO:______); DSP13.3 (SEQ ID NO:______); DSP14.1 (SEQ ID NO:______); and DSP14.3 (SEQ ID NO:______). Each siRNA was diluted in 50 μl OptiMEM® to provide a final concentration of 10 nM per well of cells in six well tissue culture plate. In a separate tube, 3 μl of Lipofectamine™ was combined with 10 μl OptiMEM®. Each solution was incubated for 7 minutes. The two solutions were then mixed and incubated at room temperature for 22 minutes. The final volume of the mixed solution was adjusted to 500 μl and then was added to the HeLa cells. Cells were transfected with the siRNAs or with annealing buffer alone. The transfected cells were incubated with siRNAs for 60 hours.

[0193] Cell lysates were prepared by extracting the cells in RIPA buffer as described in Example 1. The lysates were separated by SDS-PAGE gel and analyzed by immunoblot according to the procedures described in Examples 1 and above in Example 2 using an anti-SHP-2 murine monoclonal antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, Calif.). The levels of expression of endogenous SHP-2 decreased by 75% in the presence of SHP2.2 and by 90% in the presence of SHP2.1. The expression of SHP-2 in the siRNAs presence of DSP13.3, DSP14.1, or DSP14.3 was comparable to the level of expression observed in cells treated with buffer only.

[0194] A similar experiment was performed to determine the level of endogenous expression of DSP-3 in HeLa cells and in MDA-MB-435 cells (ATCC) in the presence of sequence specific siRNA. DSP3.1 siRNA (SEQ ID NO:1) was transfected into each cell line as described above, and the level of expression of DSP-3 polypeptide was analyzed by immunoblot (see Example 1 for immunoblot procedure to detect DSP-3). Expression of DSP-3 polypeptide decreased 70-100% in HeLa cells and decreased 100% in MDA-MB-435 cells in the presence of the specific mRNA.

[0195] Particular siRNA polynucleotide sequences that are specific for CD45, SHP2, cdc14a, cdc14b, cdc25A, cdc25B, cdc25C, PRL-3, KAP, DSP-3, and PTPϵ are provided below. The level of expression of each PTP and DSP in cells that are capable of expressing the PTP or DSP and that are transfected with any one of the following specific siRNA polynucleotides is determined according to methods and procedures described above. The siRNA sequences that are incorporated into a vector from which a hairpin vector is transcribed and/or that are transfected via liposomes according to methods described in Examples 1 and 2 are presented in the following tables. The human TCPTP target sequences were derived from a human TCPTP nucleotide sequence (see GenBank Accession No. M25393, NM 002828, NM_080422 (SEQ ID NOs:______)); the CD45 target sequences were derived from a human CD45 nucleotide sequence, (see Charbonneau et al. (SEQ ID NO:______)); the SHP-2 target sequences were derived from a human SHP-2 nucleotide sequence (see GenBank Accession No. L03535 and L07527 (SEQ ID NO:______)); the cdc14a target sequences were derived from a human cdc14a nucleotide sequence (see GenBank Accession No. AF122013 (SEQ ID NO:______)); the cdc14b target sequences were derived from a human cdc14b nucleotide sequence (GenBank Accession No. AF023158 (SEQ ID NO:______)); the cdc25A target sequences were derived from a human cdc25A nucleotide sequence (see GenBank Accession No. NM_133571 and AF527417 (SEQ ID NO:______); the cdc25B target sequences were derived from a human cdc25B nucleotide sequence (see GenBank Accession No. M81934 (SEQ ID NO:______)); the cdc25C target sequences were derived from a human cdc25C nucleotide sequence (see GenBank Accession No. NM_001790 (SEQ ID NO:______); the PRL-3 target sequences are derived from the human PRL-3 nucleotide sequence (see GenBank Accession No. NM_032611 and NM_003479 (SEQ ID

NO:______); the KAP target sequences are derived from the human KAP nucleotide sequence (see GenBank Accession No. L2711 (SEQ ID NO:______)); the DSP-3 target sequences were derived from the human DSP-3 nucleotide sequence set forth in (SEQ ID NO:778); and the PTPε target sequences were derived from the human PTPε nucleotide sequence (see GenBank Accession No. NM_006504 and NM_130435 (SEQ ID NO:______)).

[0196] siRNA polynucleotide sequences were selected using the Dharmacon siDESIGN system (Dharmacon Research). These sequences were generated using the following parameters: (1) leader sequences included dinucleotides AA, CA, TA, and GA; (2) the coding region (CR) was scanned; (4) the G+C content varied from approximately 31-63%; (5) overlaps of sequences within different 19 nucleotide sequences were permitted. These sequences were then compared to known human genome sequences using the BLAST program. Potential target sequences were eliminated if 16 or more consecutive nucleotides within the 19-nucleotide target sequence were identified in another human polynucleotide sequence. The remaining 19-nucleotide siRNA sequences are presented in the tables below. Each siRNA sequence represented in Tables 2-12 lists the sequence of the sense strand of the siRNA and its corresponding sequence identifier. For PRL-3, only one sequence (AGACCCGGUGCUGCGUUAU, SEQ ID NO:______) was identified by this method. An siRNA polynucleotide as described herein is understood to be composed of the 19 nucleotide sense strand and its complementary (or antisense) strand. In addition, a siRNA polynucleotide of the present invention typically has a dinucleotide overhang at the 3' end of each strand, which may be any two nucleotides. Accordingly, it is noted that each 21-mer sequence below contains a dinucleotide "overhang" at the 3' end, and that the invention herein should be considered to include the 19-mer polynucleotide sequences beginning at the 5' end therein as well as the 21-mer polynucleotide shown in the Tables.

TABLE 2

HUMAN CD45 siRNA POLYNCULEOTIDE SEQUENCES (POST-BLAST)

19-Nucleotide Target Sequence	Region	SEQ ID NO.
CCACCAUCACAGCGAACAC	CR	
AGCGCUGUCAUUUCAACCA	CR	
ACCACAACAAUAGCUACUA	CR	
GCUACUACUCCAUCUAAGC	CR	
AAUGCGUCUGUUUCCAUAU	CR	
AUGCGUCUGUUUCCAUAUC	CR	
UGCGUCUGUUUCCAUAUCU	CR	
ACCUUUACUUGUGAUACAC	CR	
CAGAUUUCAGUGUGGUAAU	CR	
ACCCGAACAUGAGUAUAAG	CR	
CCCGAACAUGAGUAUAAGU	CR	
CAAGUUUACUAACGCAAGU	CR	
GGAGUAAUUACCUGGAAUC	CR	
CAUGCCUACAUCAUUGCAA	CR	
AUAGUAUGCAUGUCAAGUG	CR	
UGAACGUUACCAUUUGGAA	CR	
AUGAGUCGCAUAAGAAUUG	CR	
UGAGUCGCAUAAGAAUUGC	CR	
GAAUUGCGAUUUCCGUGUA	CR	
AUUGCGAUUUCCGUGUAAA	CR	
GCCAAUCCAUGCAGAUAUU	CR	
UUAUAACCGUGUUGAACUC	CR	
UAACCGUGUUGAACUCUCU	CR	
ACGGAGAUGCAGGGUCAAA	CR	

TABLE 2-continued

HUMAN CD45 siRNA POLYNCULEOTIDE SEQUENCES (POST-BLAST)

19-Nucleotide Target Sequence	Region	SEQ ID NO.
GAUGCAGGGUCAAACUACA	CR	
ACCCAGGAAAUACAUUGCU	CR	
UGUCCAGAUUACAUCAUUC	CR	
AUGCCUUCAGCAAUUUCUU	CR	
CAGGAACCUAUAUCGGAAU	CR	
GGAACCUAUAUCGGAAUUG	CR	
ACCUAUAUCGGAAUUGAUG	CR	
GUGGAUGUUUAUGGUUAUG	CR	
GGCGACAGAGAUGCCUGAU	CR	
GAGGCCCAGUACAUCUUGA	CR	
GGCCCAGUACAUCUUGAUC	CR	
GCUACUGGAAACCUGAAGU	CR	
ACCUGAAGUGAUGAUUGCU	CR	
AGUUGACCUGAAAGACACA	CR	
ACUUAUACCCUUCGUGUCU	CR	
CUUAUACCCUUCGUGUCUU	CR	
GGAAAGACUCUCGAACUGU	CR	
ACCCAAGGAAUUAAUCUCU	CR	
CCCAAGGAAUUAAUCUCUA	CR	
UGAUUCAGGUCGUCAAACA	CR	
GGGAUGGAUCUCAGCAAAC	CR	
UCUCAGCAAACGGGAAUAU	CR	
UUCGAGCAAUAUCAAUUCC	CR	
CCUACCCUGCUCAGAAUGG	CR	

[0197]

TABLE 3

HUMAN SHP-2 siRNA POLYNCULEOTIDE SEQUENCES (POST-BLAST)

19-Nucleotide Target Sequence	Region	SEQ ID NO.
AUGGAGCUGUCACCCACAU	CR	
UGGAACAUCACGGGCAAUU	CR	
GCAAUGACGGCAAGUCUAA	CR	
AUGACGGCAAGUCUAAAGU	CR	
UGACGGCAAGUCUAAAGUG	CR	
GUCUAAAGUGACCCAUGUU	CR	
UGAUUCGCUGUCAGGAACU	CR	
CGACGUUGGUGGAGGAGAA	CR	
ACGGUUUGAUUCUUUGACA	CR	
UUCUUUGACAGAUCUUGUG	CR	
GAAUCCUAUGGUGGAAACA	CR	
AUCCUAUGGUGGAAACAUU	CR	
UCCUAUGGUGGAAACAUUG	CR	
CAGUACUACAACUCAAGCA	CR	
UUUGAGACACUACAACAAC	CR	
AACUUCUCUACAGCCGAAA	CR	
ACAUCCUGCCCUUUGAUCA	CR	
UCAUACCAGGGUUGUCCUA	CR	
UACCAGGGUUGUCCUACAC	CR	
UUUGAAACCAAGUGCAACA	CR	
AGAGUUACAUUGCCACACA	CR	
GAGUUACAUUGCCACACAA	CR	
AAACACGGUGAAUGACUUU	CR	
CUGGCCUGAUGAGUAUGCU	CR	
UGGCGUCAUGCGUGUUAGG	CR	
UGCGUGUUAGGAACGUCAA	CR	
UGACUAUACGCUAAGAGAA	CR	
CUAUACGCUAAGAGAACUU	CR	
GGUUGGACAAGGGAAUACG	CR	
GAACGGUCUGGCAAUACCA	CR	
CGGUCUGGCAAUACCACUU	CR	
AAGGUGUUGACUGCGAUAU	CR	
AGGUGUUGACUGCGAUAUU	CR	
GGUGUUGACUGCGAUAUUG	CR	

TABLE 3-continued

HUMAN SHP-2 siRNA POLYNCULEOTIDE SEQUENCES (POST-BLAST)

19-Nucleotide Target Sequence	Region	SEQ ID NO.
UAUGGCGGUCCAGCAUUAU	CR	
UGGCGGUCCAGCAUUAUAU	CR	
AACACUACAGCGCAGGAUU	CR	
ACACUACAGCGCAGGAUUG	CR	
GCGCAGGAUUGAAGAAGAG	CR	
GAGGAAAGGGCACGAAUAU	CR	
GGAAAGGGCACGAAUAUAC	CR	
GGGCACGAAUAUACAAAUA	CR	
AAACGUGGGCCUGAUGCAA	CR	
ACGUGGGCCUGAUGCAACA	CR	

[0198]

TABLE 4

HUMAN CDC14A siRNA POLYNCULEOTIDE SEQUENCES (POST-BLAST)

19-Nucleotide Target Sequence	Region	SEQ ID NO.
GCACAGUAAAUACCCACUA	CR	
CUAUUUCUCCAUCGAUGAG	CR	
ACUUGGCAAUGGUGUACAG	CR	
GGUGCCUAUGCAGUAAUCU	CR	
UCUCACCAUUCUCGACUGU	CR	
AAGGGAUUACAACAUGGAU	CR	
AGGGAUUACAACAUGGAUU	CR	
GGGAUUACAACAUGGAUUU	CR	
GAAUGGUUAUCCUCUUCAC	CR	
GCAUAAUGUGACUGCAGUU	CR	
CGCUGGCUUCGAGCACUAU	CR	
GCACACCCAGUGACAACAU	CR	
ACAUCGUGCGAAGGUUCCU	CR	
AGAACAGGGACAUUGAUAG	CR	
GAACAGGGACAUUGAUAGC	CR	
GGGACAUUGAUAGCCUGUU	CR	
CAUUGAUAGCCUGUUAUGU	CR	
CUACAGGUUUACACAUGCU	CR	
AAAUCGACCAUCCAGUGAA	CR	
AAUCGACCAUCCAGUGAAG	CR	
UCGACCAUCCAGUGAAGGA	CR	
AAAUUCUUUCUGGCCUAGA	CR	
UGUCUAUUGGUGGAAAUCU	CR	
ACGAUUUGGAGAGGUAAGU	CR	
CGAUUUGGAGAGGUAAGUU	CR	

[0199]

TABLE 5

HUMAN CDC14B siRNA POLYNCULEOTIDE SEQUENCES (POST-BLAST)

19-Nucteotide Target Sequence	Region	SEQ ID NO.
GAGACAUCCUAUAUUCCUU	CR	
AUACCAGACCGAUUUAUUG	CR	
UACCAGACCGAUUUAUUGC	CR	
GACCGAUUUAUUGCCUUCU	CR	
AAGGAUGUAUGAUGCCAAA	CR	
AGGAUGUAUGAUGCCAAAC	CR	
GGAUGUAUGAUGCCAAACG	CR	
CGGAUGCUGGCUUCGAUCA	CR	
UGCCAUUGUCAAAGAAUUC	CR	
GGGUGCCAUUGCAGUACAU	CR	
GACCUGGCUCGGUGAUUGG	CR	

TABLE 5-continued

HUMAN CDC14B siRNA POLYNCULEOTIDE SEQUENCES (POST-BLAST)

19-Nucteotide Target Sequence	Region	SEQ ID NO.
CCCGAACCGUACAGUGAUG	CR	
ACCGUACAGUGAUGAUGAC	CR	
UAGACUUCGGGCCUUGAAA	CR	
ACAAACGCUAUUCCUCUCA	CR	

[0200]

TABLE 6

HUMAN CDC25A siRNA POLYNCULEOTIDE SEQUENCES (POST-BLAST)

19-Nucleotide Target Sequence	Region	SEQ ID NO.
GGGUCUGGGCAGUGAUUAU	CR	
GCAACCACUGGAGGUGAAG	CR	
AUCCUAUGAGAAGAAUACA	CR	
UCCUAUGAGAAGAAUACAU	CR	
AAAGCUGUUGGGAUGUAGU	CR	
UUCUGAUUCUCUUGACCAU	CR	
GAAGCCAGUAAGACCUGUA	CR	
CAGCCACUUUGUCUGAUGA	CR	
AACCUUGACAACCGAUGCA	CR	
CAACCGAUGCAAGCUGUUU	CR	
ACCGAUGCAAGCUGUUUGA	CR	
CUCGGUCAGUGUUGAAGAG	CR	
ACGUUCUCAAGAGGAGUCU	CR	
GUCAACUAAUCCAGAGAAG	CR	
AGGCCCAUGAGACUCUUCA	CR	
AGGGACCUUAUAGGAGACU	CR	
GGGACCUUAUAGGAGACUU	CR	
GACUUCUCCAAGGGUUAUC	CR	
GUUUGUUAUCAUCGACUGU	CR	
CUGUCGAUACCCAUAUGAA	CR	
GAAGCCCAUUGUACCUACU	CR	
AGCCCAUUGUACCUACUGA	CR	
GCCCAUUGUACCUACUGAU	CR	
UGGCAAGCGUGUCAUUGUU	CR	
AGCGUGUCAUUGUUGUGUU	CR	
UGUGCCGGUAUGUGAGAGA	CR	
GAGAGAUCGCCUGGGUAAU	CR	
GAGAUCGCCUGGGUAAUGA	CR	
GAUCGCCUGGGUAAUGAAU	CR	

[0201]

TABLE 7

HUMAN CDC25B siRNA POLYNCULEOTIDE SEQUENCES (POST-BLAST)

19-Nucleotide Target Sequence	Region	SEQ ID NO.
AUCCUCCCUGUCGUCUGAA	CR	
UCCUCCCUGUCGUCUGAAU	CR	
UGGCGGAGCAGACGUUUGA	CR	
CGUUUGAACAGGCCAUCCA	CR	
GCCGGAUCAUUCGAAACGA	CR	
UCAUUCGAAACGAGCAGUU	CR	
GUCUAUGCCGGAUGGAUUU	CR	
UGCCGGAUGGAUUUGUCUU	CR	
AAAGGACCUCGUCAUGUAC	CR	
AAUCACUGUGUCACGAUGA	CR	
AUCACUGUGUCACGAUGAG	CR	
GAGCUGAUUGGAGAUUACU	CR	
GCUGAUUGGAGAUUACUCU	CR	

TABLE 7-continued

HUMAN CDC25B siRNA POLYNCULEOTIDE SEQUENCES (POST-BLAST)		
19-Nucleotide Target Sequence	Region	SEQ ID NO.
CUCUAAGGCCUUCCUCCUA	CR	
CAGACAGUAGACGGAAAGC	CR	
AGCACCAAGACCUCAAGUA	CR	
GAAACGAUGGUGGCCCUAU	CR	
AACGAUGGUGGCCCUAUUG	CR	
CGCCGAGAGCUUCCUACUG	CR	

[0202]

TABLE 8

HUMAN CDC25C siRNA POLYNCULEOTIDE SEQUENCES (POST-BLAST)		
19-Nucleotide Target Sequence	Region	SEQ ID NO.
GAACUCCAGUGGGCAAAUU	CR	
UUUAGCUGGGAUGACAAUG	CR	
UUCAAGGACAACACAAUAC	CR	
ACACAAUACCAGAUAAAGU	CR	
CACAAUACCAGAUAAAGUU	CR	
GGAAGGGCUUAUGUUUAAA	CR	
CACCAAGAUCUGAAGUAUG	CR	
AGUAUGUCAACCCAGAAAC	CR	
GUAUGUCAACCCAGAAACA	CR	
UGUCAUUGAUUGUCGCUAU	CR	
UUGAUUGUCGCUAUCCAUA	CR	
UUGUCGCUAUCCAUAUGAG	CR	
UCCAGGGAGCCUUAAACUU	CR	
GGGAGCCUUAAACUUAUAU	CR	
GUCAGGAAGAACUGUUUAA	CR	
AGAAGCCCAUCGUCCCUUU	CR	
GAAGCCCAUCGUCCCUUUG	CR	
AGCCCAUCGUCCCUUUGGA	CR	
CACCCAGAAGAGAAUAAUC	CR	
UUGUACUACCCAGAGCUAU	CR	
CUACCCAGAGCUAUAUAUC	CR	
CCCAGAGCUAUAUAUCCUU	CR	
UAUAUGGAACUGUGUGAAC	CR	
UAUGGAACUGUGUGAACCA	CR	
CAGAGCUACUGCCCUAUGC	CR	
GAGCUACUGCCCUAUGCAU	CR	
GCUACUGCCCUAUGCAUCA	CR	

[0203]

TABLE 9

HUMAN KAP siRNA POLYNCULEOTIDE SEQUENCES (POST-BLAST)		
19-Nucleotide Target Sequence	Region	SEQ ID NO.
GAUGAAGAGCCUAUUGAAG	CR	
AGAUGAACAGACUCCAAUU	CR	
GAUGAACAGACUCCAAUUC	CR	
UCACCCAUCAUCAUCCAAU	CR	
GAGCUUACAACCUGCCUUA	CR	
CACUGCUAUGGAGGACUUG	CR	
UCACCAGAGCAAGCCAUAG	CR	
CCAGAGCAAGCCAUAGACA	CR	
CAGCCUGCGAGACCUAAGA	CR	
GUUUCGGGACAAAUUAGCU	CR	
AAUUAGCUGCACAUCUAUC	CR	

TABLE 9-continued

HUMAN KAP siRNA POLYNCULEOTIDE SEQUENCES (POST-BLAST)		
19-Nucleotide Target Sequence	Region	SEQ ID NO.
AUUAGCUGCACAUCUAUCA	CR	
UUAGCUGCACAUCUAUCAU	CR	

[0204]

TABLE 10

HUMAN DSP-3 siRNA POLYNCULEOTIDE SEQUENCES (POST-BLAST)		
19-Nucleotide Target Sequence	Region	SEQ ID NO.
GAGACGCGGAACAAUUGAG	CR	
AGAACAAGGUGACACAUAU	CR	
GAACAAGGUGACACAUAUU	CR	
GCAGCGGAUUCACCAUCUC	CR	
GCGGAUUCACCAUCUCAAA	CR	
CACUGGUGAUCGCAUACAU	CR	
GUAUCGGCAGUGGCUGAAG	CR	

[0205]

TABLE 11

HUMAN PTP EPSILON siRNA POLYNCULEOTIDE SEQUENCES (POST-BLAST)		
19-Nucleotide Target Sequence	Region	SEQ ID NO.
GAUCCGCCGACGACUGCAA	CR	
GUUUCGGGAGGAGUUCAAC	CR	
AUGACCAUUCUAGGGUGAU	CR	
CCAUUCUAGGGUGAUUCUG	CR	
CAUAGAUGGUUACAAAGAG	CR	
AACAGGAAACGGUUAACGA	CR	
GGAAACGGUUAACGACUUC	CR	
CCAUCGUCAUGUUAACAAA	CR	
CUACACCAUCCGGAAGUUC	CR	
UCCGGAAGUUCUGCAUACA	CR	
GAAAGUAAAGACGCUCAAC	CR	
GCGCCCUCAGAUGGUUCAA	CR	
CGGAUAUGCAGUACACGUU	CR	
CCACCCACUUCGACAAGAU	CR	
CAAAUGUCCGGAUCAUGAA	CR	
CAUGAGGACGGGCAACUUG	CR	
UGACUUCAACCGAGUGAUC	CR	
ACCGAGUGAUCCUUUCCAU	CR	
AGAAUACACAGACUACAUC	CR	
GACUACAUCAACGCAUCCU	CR	
UCAACGCAUCCUUCAUAGA	CR	
CACACGGUUGAGGACUUCU	CR	
AAUCCCACACUAUCGUGAU	CR	
AUCCCACACUAUCGUGAUG	CR	
ACCGAGGGCUCAGUUACUC	CR	
CCGAGGGCUCAGUUACUCA	CR	
CUCAUGGAGAAAUAACGAU	CR	
UGGAGAAAUAACGAUUGAG	CR	
GCCAUCAGUAUACGAGACU	CR	
UCAGUAUACGAGACUUUCU	CR	
GGGCAAAGGCAUGAUUGAC	CR	
GCUGGGCGAACAGGUACAU	CR	
CUUCAGAGACCACAUAUGG	CR	

EXAMPLE 3

Decreased Activation of JNK in the Presence of siRNA Specific for DSP-3

[0206] This Example describes the effect on JNK activation by sequence-specific siRNA interference of DSP-3 polypeptide expression.

[0207] HeLa cells were transfected with 60 pmoles of DSP3.1 siRNA (SEQ ID NO:1) or 60 pmoles CD45.2 (SEQ ID NO:13) as described in Example 1. After the incubation following transfection, cells were stimulated with 10 ng/ml TNF-α or 10 ng/ml EGF for 10 minutes or with 500 mM sorbitol for 30 minutes, which are known stimulators of the JNK signal transduction pathway (WO 01/21812; Shen et al. *Proc. Natl. Acad. Sci.* 98:13613-18 (2001)). After the stimulators were decanted, the 6-well plate of cells was frozen. The cells were treated with 0.5 ml Extraction Buffer (20 mM Tris, pH 8, 136 mM NaCl, 50 mM NaF; 1 mM V04; 0.2 mM EDTA, 0.2 mM EGTA, 20 nM Calyculin, 10% glycerol, 0.5% nonidet P40, 1 μg/ml of aprotinin, pepstatin, and leupeptin; and 1 mM Benzamidine) (4° C.). When the cells had partially thawed, the wells of the plates were scraped and the cells were collected. The wells were washed 3× with Extraction Buffer and the washes were combined with the cells. After centrifugation of the extracted cells, the supernatants were decanted. The protein concentration of each extract was determined by the Bradford protein assay. Volumes of the different extracts were adjusted with Extraction Buffer to the concentration of the extract having the lowest protein concentration.

[0208] JUN, a substrate of JNK, conjugated to glutathione (GSH) (GST-cJUN) (Shen et al., supra) in 20 mM Tris, pH 7.2, 1 nM EDTA, and 150 mM NaCl was combined with 200-250 μl of Glutathione-Sepharose (Amersham Biosciences, Piscataway, N.J.). After mixing for 45 minutes at 4° C., the conjugated sepharose beads were washed twice in Extraction Buffer and then resuspended in 1 ml of Extraction Buffer.

[0209] cJUN-Sepharose (20 μl) was added to each cell extract sample. The mixtures were gently mixed for 2 hours at 4° C., followed by one wash in 1 ml Extraction Buffer and once in 1 ml kinase buffer (20 mM Pipes, pH 7.2, 10 mM $MgCl_2$, 1 mM DTT, 0.1% Triton X-100, and 1 MM sodium vanadate). The mixtures were centrifuged and the pellets were kept on ice. ATP mix (300° C./ml of [γ-^{32}P]ATP (3000 Ci/mmole) in kinase buffer) was incubated in a heat block to bring the solution to 30° C. ATP mix (15 μl) was added to each cold cJUN-Sepharose pellet at time intervals of 20 seconds. After the ATP mix was added, each sample was vortexed gently for 5 seconds and then placed in the 30° C. heat block. Each sample was gently mixed again for 5 seconds at 20-second intervals. After 20 minutes, the reactions were stopped at 20-second intervals with 15 μl 2×SDS-PAGE sample buffer. The samples were immediately heated at 100° C. for 5 minutes, then mixed and frozen at −20° C. The extracts were thawed and applied to 8-16% NOVEX® gels. After electrophoresis, the gels were dried and the cJUN band was cut from the gel and the radioactivity was counted (Cerenkov measurement). As shown in **FIGS. 3 and 4**, JNK activation as measured by the presence of phosphorylated JUN was mediated less by cells transfected with siRNA specific for DSP-3 than in cells transfected with a non-specific siRNA.

[0210] Because EGF induces a signaling pathway involving the ERK MAP kinase family, the effect on ERK phosphorylation in HeLa cells transfected with DSP-3 specific siRNA was determined. Transfection of HeLa cells and stimulation of the JNK signaling pathway was performed as in the previous experiment. Additional transfected cell cultures were stimulated with anisomycin. Phosphorylation of ERK was determined in a similar manner as described above for cJUN except that after electrophoresis of the cell extract samples, the proteins separated in the gel were transferred to a PVDF membrane. The immunoblot was probed with an anti-phospho-ERK antibody (1:1000) followed by incubation with the appropriate HRP-conjugated reagent and detection by chemiluminescence. As shown in **FIG. 5**, phosphorylation of ERK induced by stimulation of the cells with EGF and sorbitol was not affected by interference of DSP-3 polypeptide expression by specific siRNA DSP3.1.

EXAMPLE 4

Interference of Expression and Function of Cell Division Cycle Proteins by Specific siRNA

[0211] This example describes interference of expression of cell division cycle (cdc) proteins, cdc14a, cdc14b, and cdc25A, cdc25B, and cdc25C polypeptides by sequence specific siRNA polynucleotides. The effect on the function of these polypeptides in the presence of siRNA was also determined.

[0212] Interference with Cell Division Cycle Protein Expression by Specific siRNA

[0213] Two siRNA sequences that were specific for cdc14a polynucleotide (SEQ ID NO:33) encoding a cdc14a polypeptide (SEQ ID NO: 34) and two siRNA sequences specific for cdc14b polynucleotide (SEQ ID NO:35) encoding a cdc14b polypeptide (SEQ ID NO:36) were designed using the criteria described in Example 1. Recombinant expression vectors containing polynucleotide sequences encoding FLAG®-tagged cdc14a polypeptide and FLAG®-tagged cdc14b polypeptide were prepared essentially according to methods described in Example 2 with the following exceptions. 293-HEK cells were cultured in 35 mm culture dishes and were transfected with FLAG vectors at a concentration of 1 μg per well. 293-HEK cells were co-transfected with FLAG®-tagged cdc14a expression vector and the following siRNAs at 20 nM per well: cdc14a.2 (5'-caucugugagaacaccgaatt-3', SEQ ID NO:______); cdc14a.3 (5'-cuuggcaaugguguacagatt-3', SEQ ID NO:______); cdc14a.4, SEQ ID NO:______), cdc14a.5 (5'-gcacaguaaauacccacuatt-3', SEQ ID NO:______); DSP3.1 (SEQ ID NO:______); DSP3.2 (SEQ ID NO:______); cdc14b.3 (5'-caagcaaaugcugccuucctt-3', SEQ ID NO:______); cdc14b.4 (5'-gagccagacuugaaaguggtt-3', SEQ ID NO:______); MKP.2 (SEQ ID NO:______); and CD45.3 (negative control). Controls included 293-HEK cells that were not transfected with any vector or siRNA and 293-HEK cells transfected with FLAG®-tagged cdc14a in the presence of siRNA annealing buffer. The level of expression in each sample was analyzed by immunoblot as described in Example 2 using an anti-FLAG® antibody. As shown in **FIG. 6**, specific siRNAs, cdc14a.2, cdc14a.3, and cdc14a.5 interfered with expression of cdc14a polypeptide most effectively.

[0214] Specificity of cdc14a.3 siRNA for interfering with expression of cdc14a and not other dual specificity phos-

phatases was shown by co-transfecting cdc14a.3 siRNA with FLAG®-tagged cdc14a (1 μg per 35 mm well of cells), FLAG®-tagged DSP-3, FLAG®tagged cdc14b, and FLAG®-tagged DSP-11. A FLAG® recombinant expression construct containing a polynucleotide sequence (SEQ ID NO:______) encoding a DSP-3 polypeptide (SEQ ID NO:______) was prepared as described for constructing other FLAG vectors. 293-HEK cell transfections and analysis of polypeptide expression levels were performed as described in Example 2. **FIG. 7** shows that siRNA cdc14a.3 interfered with expression of only the cdc14a dual specificity phosphatase.

[0215] 293-HEK cells were co-transfected with FLAG®-tagged cdc14b expression vector (2 μg/35 mm well) and the following siRNAs at 20 nM per well: cdc14b.3 (SEQ ID NO:______); cdc14b.4 (SEQ ID NO:______); cdc14a.3 (SEQ ID NO:______)); cdc14a.5 (SEQ ID NO:______)); DSP3.1 (SEQ ID NO:______); DSP3.2 (SEQ ID NO:______); MKP.2 (SEQ ID NO:______); and CD45.3. Controls included 293-HEK cells that were not transfected with any vector or siRNA and 293-HEK cells transfected with FLAG®-tagged cdc14b in the presence of siRNA annealing buffer. The level of expression in each sample was analyzed by immunoblot as described in Example 2 using an anti-FLAG® antibody. As shown in **FIG. 8**, only specific siRNAs, cdc14b.3 and cdc14b.4 interfered with expression of cdc14b polypeptide.

[0216] Specificity of cdc14b.3 and cdc14b.4 siRNAs for interfering with expression of cdc14b and not other dual specificity phosphatases was shown by co-transfecting the siRNAs with FLAG®-tagged cdc14b (2 μg per 35 mm well), FLAG®-tagged DSP-3, and FLAG®-tagged DSP-11. Cells transfected with FLAG®-tagged DSP-3 and FLAG®-tagged DSP-11 were also co-transfected with cdc14a.5 siRNA. 293-HEK cell transfections and analysis of polypeptide expression levels were performed as described in Example 2. **FIG. 9** shows that cdc14b.3 and cdc14b.4 siRNAs interfered with expression of only the cdc14b dual specificity phosphatase.

[0217] Expression of cdc14b polypeptide co-transfected with cdc14b.4 siRNA in HeLa cells was analyzed by immunocytochemistry. HeLa cells were co-transfected with a cdc14b recombinant expression vector and siRNA. Expression of cdc14b was detected by standard immunocytochemistry methods. As shown in **FIG. 10**, cdc14b.4 siRNA interfered with expression of cdc 14b polypeptide (top and bottom right panels).

[0218] The efficacy of RNAi against FLAG®-tagged Cdc25A expression in 293-HEK cells was also determined. Cells were co-transfected with a FLAG®-Cdc25A expression construct (prepared as described in Example 2) and specific siRNAs 25A.1, 25A.2, 25A.3, and 25A.4 (20 nM) and non-specific siRNAs (25B.1-0.4 and 25C.1-0.4). The level of expression of Cdc25A was determined by immunoblotting with an anti-FLAG® antibody. Only siRNA 25A.2 (5'-gaggagccauucugauucutt-3' (SEQ ID NO:______)) effectively inhibited expression of Cdc25A.

[0219] The effect of RNAi on endogenous expression of Cdc25B and Cdc25C was examined in HeLa cells. The experiments were performed essentially as described in Example 2, except that HeLa cells were exposed to 10 nM siRNA polynucleotides for 48 hours. Four siRNAs specific for Cdc25A: 25A.1, 25A.2, 25A.3, and 25A.4 (20 nM); four siRNAs specific for Cdc25B: 25B.1, 25B.2, 25B.3, and 25B.4 (20 nM); and four siRNAs specific for Cdc25C: 25C.1, 25C.2, 25C.3, and 25C.4 (20 nM) were transfected into HeLa cells and expression was analyzed by immunoblotting cell lysates separated by SDS-PAGE using a Cdc25B antibody (Santa Cruz Biotechnololgy, Cat. No. c-20) and a Cdc25C antibody (Santa Cruz Biotechnololgy, Cat. No. h-85). The level of expression of Cdc25B was decreased 40-50% in HeLa cells transfected with siRNA cdc25B.2 (5'-aggcggcuacaaggaguuctt-3' (SEQ ID NO:______)), and 50-60% in cells transfected with cdc25B.4 siRNA 5'-gaugccauggaagcccacatt-3' (SEQ ID NO:______)). In HeLa cells transfected with siRNAs specific for Cdc25C, the level of expression of Cdc25C decreased 90% in HeLa cells transfected with cdc25C.1 (5'-cugccacucagcuuaccactt-3' (SEQ ID NO:______)); decreased 70-80% in cells transfected with cdc25C.3 (5'-cccagaaacaguggcugccctt-3' (SEQ ID NO:______)); and decreased 70-80% in cells transfected with Cdc25C.4 (5'-aggcggcuacagagacuuctt-3' (SEQ ID NO:______)).

[0220] The ability of cancer cell lines to mediate RNA interference was examined by co-transfecting several cancer cell lines with a FLAG® cdc14b expression construct and specific siRNAs. The cell lines included SW620 (colon cancer); MCF7 (breast cancer); HS578T (breast cancer); MDA MB 231 (breast cancer); and T47D (breast cancer) (ATCC, NCI 60 panel). The FLAG® cdc14b expression construct (1–2 μg) was co-transfected with 20 nM of 14b.3 siRNA (SEQ ID NO:______); 14b.4 siRNA (SEQ ID NO:______); or MKP.2 siRNA (SEQ ID NO:______) (non-specific control) into each cell line as described in Example 2. The level of expression was analyzed by immunoblotting with an anti-FLAG® antibody according to the method described in Example 2. Expression of cdc14b was decreased in each of the five cell lines that were co-transfected with a cdc14b specific siRNA polynucleotide.

[0221] Effect of CDC-Specific siRNA on Cell Proliferation

[0222] Proliferation of cancer cells in the presence of siRNA polynucleotides specific for cdc14a, cdc14b, and Cdc25A, Cdc25B, and Cdc25C was determined. Cell proliferation was assessed according to a quantitative metabolic assay that measures the enzymatic conversion by cellular dehydrogenase in viable cells of a yellow tetrazolium salt (methylthiazoletetrazolium (MTT)) to purple formazan crystals. MDA-MB-231, SW620, and HeLa cell lines were transfected according to the procedures described in Examples 1 and 2 with the following siRNA polynucleotides (5 nM): cdc14a.3 (5'-cuuggcaaugguguacagatt-3' (SEQ ID NO:______)); cdc14a.5 (5'-gcacaguaaauacccacuatt-3' (SEQ ID NO:______)); cdc14b.3 (5'-caagcaaaugcugccuucctt-3' SEQ ID NO:______); cdc14b.4 (5'-gagccagacuugaaagguggtt-3' SEQ ID NO:______); cdc25A.2 (SEQ ID NO:______); cdc25B.4 (SEQ ID NO:______); cdc25C.1 (SEQ ID NO:______). The transfected cells were seeded at in a tissue culture plate and maintained for 5 days. A MTT assay was performed according to manufacturer's instructions (ATCC MTT Cell Proliferation Assay Kit, Cat. NO. 30-1010K, ATCC). The MTT-containing media was removed from the wells and was added to solubilize the formazan. The amount of formazan formed was determined by measuring absorbance at 570 m. Compared to the buffer only control, a

significant decrease in proliferation was observed in MDA-MB-231 cells transfected with cdc14a.3, cdc14a.5, cdc14b.3, cdc14b.4, and cdc25B.4, and in HeLa cells transfected with cdc14a.3, cdc14a.5, cdc14b.4, and cdc25B.4. A significant decrease in cell proliferation of SW620 cells transfected with cdc14a.3 or cdc14a.5 was also observed.

[0223] Effect of CDC-Specific siRNA on Proapoptotic Signaling

[0224] Poly(ADP-ribose) polymerase (PARP) is a nuclear DNA binding protein that participates in genome repair, DNA replication, and the regulation of transcription. Cleavage of PARP (approximately 115 kDa) by members of the caspase family into polypeptide fragments of approximately 85 kDa and 25 kDa prevents PARP from performing its normal repair functions and appears to be an early event in apoptotic cell death. The cleaved PARP fragments can be detected by a variety of immunodetection methods.

[0225] HeLa cells were transfected with cdc14a.5 (SEQ ID NO:______); cdc14b.4 (SEQ ID NO:______); cdc25A.2 (SEQ ID NO:______); cdc25B.4 (SEQ ID NO:______); and cdc25C.1 (SEQ ID NO:______) at a concentration of 10 nM. After incubating the transfected cells for at 37° C., cell lysates were prepared and an immunoblot performed an antibody that that specifically binds to cleaved PARP and an antibody that binds to PARP (Cell Signaling Technology, Beverly, Mass.). The results are presented in **FIG. 24**. The data indicated that inhibiting expression of cdc14a by specific siRNA induces proapoptotic signaling to a greater extent than inhibition of expression of the other cell division cycle proteins.

EXAMPLE 5

Interference of PTP-1B and TC-PTP Expression by Specific siRNA

[0226] This Example describes interference with expression of two protein tyrosine phosphatases, PTP-1B and TC-PTP, using sequence specific siRNA polynucleotides.

[0227] Interference of Endogenous Expression of Murine PTP-1B in Mouse Fibroblasts by Sequence Specific siRNA Polynucleotides

[0228] Three siRNA sequences that were specific for murine PTP-1B polynucleotide (GenBank Acc. No. NM_011201, SEQ ID NO:______) encoding a murine PTP-1B polypeptide (GenBank Acc. No. NM_011201, SEQ ID NO:______) and one siRNA sequences specific for human PTP-1B polynucleotide (GenBank Ace. No. NM_02827, SEQ ID NO:______) encoding a human PTP-1B polypeptide (GenBank Ace. No. NM_02827, SEQ ID NO:______) were designed using the criteria described in Examples 1 and 2. Mouse C57B16 #3 cells, clones 3 and 10, were maintained in cell culture according to standard cell culture methods. Each C57B16 #3 clone was transfected with 200 nM of the following siRNAs: mPTP1B.1 (SEQ ID NO:______), mPTP1B.2 (SEQ ID NO:______), mPTP1B.3 (SEQ ID NO:______), and hPTP1B.1 (SEQ ID NO:______). Each siRNA was diluted in 50 μl O_{PTI}MEM® to provide a final concentration of 200 nM per well. In a separate tube, 3 μl of Lipofectamine™ was combined with 10 μl O_{PTI}MEM®. Each solution was incubated for 7 minutes. The two solutions were then mixed and incubated at room temperature for 22 minutes. The final volume of the mixed solution was adjusted to 100 μl and then the C57B16 #3 cells were added. Cells were transfected with the specific siRNAs, the human PTP1B siRNA, or annealing buffer alone. The transfected cells were incubated with siRNAs for six days.

[0229] Cell lysates were prepared by extracting the cells in ELISA extraction buffer (50 mM Tris-HCl, pH 7.5 (room temperature); 2 mM EDTA, pH 7-8; 1 mM phosphate (polyphosphate); 1 mM NaVO4 (monomeric), pH 10; 0.1% Triton X-100; Protease Inhibitor Cocktail set III, (Calbiochem, San Diego, Calif., catalog #539134)). The lysates were separated by SDS-PAGE gel and analyzed by immunoblot according to the procedures described in Examples 1 and 2 using an anti-PTP1B murine monoclonal antibody (Dr. Ben Neel, Harvard University, Cambridge, Mass.). As shown in **FIG. 11**, the levels of expression of endogenous PTP1B were decreased only in C57B16 cells transfected with the murine PTP1B sequence specific siRNAs.

[0230] The effect of RNAi on endogenous expression of murine PTP1B in a second murine cell line was examined. Mouse PTP1B:3T31R fibroblasts were transfected with 20 nM mPTP1B1.1 (SEQ ID NO:______); mPTP1B1.6 (SEQ ID NO:______); and mPTP1B1.8 (SEQ ID NO:______) according to the method described above. The level of murine PTP1B expression in the cells transfected with mPTPB11.1 decreased approximately 80% compared with cells transfected with a non-specific siRNA (hPTP1B1.3 (SEQ ID NO:______)); cells transfected with mPTP1B1.6 decreased approximately 40%; and cells transfected with mPTP1B1.8 decreased approximately 60%.

[0231] Interference with Murine PTP1B Expression by siRNA in Co-Transfection Assays

[0232] A recombinant expression construct was prepared that encodes wild-type murine PTP1B (mPTP1B) (GenBank Accession No. NM_011201, SEQ ID NOs:______ and ______). The following oligonucleotide primers were used for the wild-type construct. The sequences of the BamHI and EcoRI restriction sites are underlined.

```
mPTP1B-sense (mPTP1B 5'BamHI)
                                 (SEQ ID NO:___)
5'-GGGGGGGATCCATGGAGATGGAGAAGGAGTTCGAGG-3'

mPTP1B anti sense (mPTP1B 3'EcoRI)
                                 (SEQ ID NO:___)
5'-GGGGGAATTCTCAGTGAAAACACACCCGGTAGCAC-3'
```

[0233] Vector pCMVTag2B (Stratagene, La Jolla, Calif.) was digested with restriction endonuclease BamHI (New England Biolabs, Beverly, Mass.) for 3 hours at 37° C. The digested vector was then incubated with Klenow polymerase (New England Biolabs) for 15 minutes at 25° C. to fill in the recessed 3' termini, followed by an incubation of 30 minutes at 37° C. with calf intestinal phosphatase (New England Biolabs). The GATEWAY™ Reading Frame Cassette B (Invitrogen Life Technologies, Carlsbad, Calif.) was inserted into the pCMVTag2B vector by ligation with T4 DNA ligase (Invitrogen Life Technologies) overnight at 16° C. according to the supplier's instructions. DB3.1™ competent *E. Coli* cells were transformed with the ligated vector (GWpCMVTag2) and DNA was isolated by standard molecular biology methods.

[0234] Vectors for expression of mPTP1 B wild type were prepared as follows. The mPTP1B construct was subcloned into a GATEWAY™ entry vector pENTR3 C™ (Invitrogen Life Technologies) by digesting 20 μl of the mPTP1B cDNA or 20 μl of the pENTR3C™ vector with 1 μl of BamHI (New England Biolabs); 1 μl of EcoRI (New England Biolabs); 5 μl 10×EcoRI buffer (New England Biolabs); 5 μl 10×BSA (New England Biolabs); and 18 μl distilled water for 3 hours at 37° C. Digested DNA was run on a 1% agarose gel, digested bands were excised, and the DNA was gel-purified using a QIAGEN Gel Extraction kit (QIAGEN, Inc., Valencia, Calif.). Four microliters of the mPTP1B cDNA was ligated into 2 μl of the pENTR3C™ vector overnight at 16° C. with 1 μl 10× Ligation Buffer (Invitrogen Life Technologies), 1 μl T4 DNA Ligase (4U/μl) (Invitrogen, Carlsbad, Calif.), and 2 μl distilled water. The construct was transformed into LIBRARY EFFICIENCY® DH5α™ cells. The FLAG® epitope-tagged mPTP1B construct was prepared by cloning the pENTR3 C™ mPTP1B WT construct into the GWpCMVTag2 vector. The pENTR3C™ construct containing the mPTP1B polynucleotide was linearized by digesting the construct with Vsp I (Promega Corp., Madison, Wis.) at 37° C. for 2 hours. The DNA was purified using a QIAGEN PCR Purification kit (QIAGEN, Inc.). Three microliters (100 ng/μl) of the GWpCMVTag2 vector were combined in a GATEWAY™ LR reaction with 6 μl linearized pENTR3C™ mPTP1B WT, 3 μl TE buffer, 4 μl Clonase™ Enzyme, and 4 μl LR reaction buffer (Invitrogen Life Technologies) for 1 hour at room temperature. After addition of Proteinase K (Invitrogen Life Technologies) to the reaction for 10 minutes, LIBRARY EFFICIENCY® DH5α™ cells were transformed with the expression construct.

[0235] The murine PTP1B expression vector (0.5 μg) was co-transfected with 20 nM murine PTP1B sequence-specific siRNA polynucleotides into PTP1B knockout mouse fibroblasts (PTP1B KO mouse embryonic fibroblasts were prepared from 13-day embryos from PTP1B knock out mice to establish the cell line, which was then transfected with human insulin receptor (1BKO+HIR) (HIR, Julie Moyers, Eli Lilly and Company, Indianapolis, Ind.)). Transfections were performed as described in Example 1. After incubating the transfected cells for 18 hours at 37° C., cell lysates were prepared, separated by 4-12% SDS-PAGE, and immunoblotted using the anti-PTP1B murine monoclonal antibody (see above). The results are summarized in Table 13.

[0236] Interference with Rat PTP1B Expression by siRNA in Co-Transfection Assays

[0237] A co-transfection assay was performed as described above in which 1BKO+HIR mouse fibroblasts were co-transfected with an expression vector containing the sequence encoding the peptide FLAG® in frame with a nucleotide sequence (SEQ ID NO:______) that encoded a rat PTP1B polypeptide (SEQ ID NO:______) (GenBank Accession No. NM_102637) and a sequence specific siRNA, rPTP1B1.1 (5'-agaagaaaaagagaugguctt-3' (SEQ ID NO:______)) (20 nM). Additional rat PTP1B specific siRNA polynucleotides examined in the co-transfection assay included rPTP1B1.2 (5'-cggauggugggguggagguctt-3' (SEQ ID NO:______)); rPTP1B1.3 (5'-uggcaagugcaaggagcuctt-3' (SEQ ID NO:______)); and rPTP1B1.4 (5'-cuacaccaccuggccugactt-3' (SEQ ID NO:______)). The level of expression of the rat PTP1B polypeptide was determined by immunoblotting cell lysates with an anti-human PTP1B antibody that also specifically binds to rat PTP1B ((PHO2, Oncogene Research Products™, Inc. San Diego, Calif.). Expression of rat PTP1B decreased approximately 50% in cells transfected with rPTP1B1.1.

[0238] Interference with Human PTP-1B Expression by siRNA in Co-Transfection Assays

[0239] Human PTP1B encoding sequence was cloned into a Pmt vector according to standard molecular biology procedures (see Flint et al., *EMBO J.* 12:1937-46 (1993)). 1BKO+HIR cells were co-transfected with the human PTP-1B expression vector and siRNA polynucleotides (20 nM) specific for human PTP-1B sequences overnight using Lipofectamine 2000. Cells were lysed as described above, and the lysates were separated by 4-12% SDS-PAGE and transferred onto a PDVF membrane. The level of expression of human PTP-1B was determined by immunoblotting with an anti-human PTP-1B antibody (PHO2, Oncogene Research Products™, Inc. San Diego, Calif.). Interference with expression of human PTP-1B was observed with four siRNA polynucleotides as indicated in Table 14.

TABLE 12

siRNA INTERFERENCE WITH MURINE PTP-1B EXPRESSION IN CO-TRANSFECTION ASSAYS

Target	siRNA Sequence (SEQ ID NO)	siRNA Name	Related SEQ ID NO:	Decrease in Expression
Murine PTP1B	5'-gaagcccagaggagcuauatt-3'	mPTP1B1.1		95%
	5'-cuacaccacauggccugactt-3'	mPTP1B1.2		Not analyzed
	5'-gacugccgaccagcugcgctt-3'	mPTP1B1.3		Not analyzed
	5'-gguaccgagaugucagccctt-3'	mPTP1B1.4		25%
	5'-ugacuauaucaaugccagctt-3'	mPTP1B1.5		Not analyzed
	5'-agaagaaaaggagaugguctt-3'	mPTP1B1.6		80%
	5'-cgggaagugcaaggagcuctt-3'	mPTP1B1.7		Not analyzed
	5'-ggaucaguggaaggagcuctc-3'	mPTP1B1.8		80%

TABLE 13

siRNA INTERFERENCE WITH HUMAN PTP-1B EXPRESSION IN CO-TRANSFECTION ASSAYS

Target	siRNA Sequence (SEQ ID NO)	siRNA Name	Related SEQ ID NO:	Decrease in Expression
Human PTP1B	5'-cuauaccacauggccugactt-3'	hPTP1B1.1		Not analyzed
	5'-gcccaaaggaguuacauuctt-3'	hPTP1B1.2		>95%
	5'-ggaagaaaaaggaagccccctt-3'	hPTP1B1.3		>95%
	5'-caaugggaaaugcagggagtt-3'	hPTP1B1.4		>95%
	5'-ggaucaguggaaggagcuutc-3'	hPTP1B1.5		>95%

[0240] Interference of Endogenous Expression of Human PTP-1B by siRNA

[0241] The effect of sequence specific siRNA on endogenous expression of human PTP-1B was examined in two different cell lines. HeLa cells were transfected as described above with HPTP1B1.1, hPTP1B1.2, hPTP1B1.3, hPTP1B1.4, and hPTP1B1.5 at 20 nM using Lipofectamine 2000, and after three days, the level of expression of PTP1B was analyzed by immunoblot. No significant decrease in expression of human PTP-1B was observed in HeLa cells transfected with the siRNA hPTP1B1.1. In HeLa cells transfected with hPTP1B1.2 and hPTP1B1.4, the level of expression of human PTP-1B decreased 80%, and in cells transfected with hPTP1B1.3, the level of expression decreased 90%. Endogenous expression of human PTP-1B in the second cell line, 293-HEK-HIR, (gift from Julie Moyers, Eli Lilly and Company) transfected with sequence specific siRNAs hPTP1B1.2, hPTP1B1.3, hPTP1B1.4, hPTP1B1.5 (20 nM) was reduced by 90%.

[0242] Interference with Expression of Murine TCPTP by siRNA in Co-Transfection Assays

[0243] A co-transfection assay was performed in which 1BKO+HIR murine fibroblasts were co-transfected as described above with an expression vector comprising a polynucleotide sequence (SEQ ID NO:______) encoding murine TCPTP (SEQ ID NO:______) and siRNA mTCPTP1.1 (5'-guugucaugcuaaaccgaact-3' (SEQ ID NO:______)) (1 nM) or mTCPTP1.2 (5'-cagaacagagugaugguugag-3' (SEQ ID NO:______)) (20 nM). The level of TCPTP expression was determined by immunoblotting with an anti-human TCPTP antibody (Curt Diltz, CEPTYR, Inc.). The siRNA mTCPTP1.2 did not interfere with expression of murine TCPTP. Expression of murine TCPTP decreased more than 95% in cells transfected with siRNA, mTCPTP1.1.

[0244] Interference with Expression of Human TCPTP by siRNA in Co-Transfection Assays

[0245] Co-transfection assays were performed essentially as described above for PTP1B expression analysis to determine siRNA inhibition of human TCPTP expression. A recombinant expression construct was prepared that encodes wild-type human TC45. The following oligonucleotide primers were used for the wild-type construct. The sequences of the BamHI and EcoRi restriction sites are underlined.

```
Human TC45 sense (TC45 5'BamHI)
5'-GGGGGGATCCATGCCCACCACCATCGAGCGGGAGTT-3'              (SEQ ID NO___)

Human TC45 antisense (TC45 3'EcoRI)
5'-GGGGAATTCTTAGGTGTCTGTCAATCTTGGCCTTTTTCTTTTTCGTTCA-3'  (SEQ ID NO:___)
```

[0246] Vector pCMVTag2B (Stratagene, La Jolla, Calif.) was digested with restriction endonuclease BamHI (New England Biolabs, Beverly, Mass.) for 3 hours at 37° C. The digested vector was then incubated with Klenow polymerase (New England Biolabs) for 15 minutes at 25° C. to fill in the recessed 3' termini, followed by an incubation of 30 minutes at 37° C. with calf intestinal phosphatase (New England Biolabs). The GATEWAY™ Reading Frame Cassette B (Invitrogen Life Technologies) was inserted into the pCMVTag2B vector by ligation with T4 DNA ligase (Invitrogen Life Technologies) overnight at 16° C. according to the supplier's instructions. DB3.1™ competent *E. coli* cells were transformed with the ligated vector (GWpCMVTag2) and DNA was isolated by standard molecular biology methods.

[0247] Vectors for expression of TC45 wild type were prepared as follows: The TC45 construct was subcloned into a GATEWAY™ entry vector pENTR3C™ (Invitrogen Life Technologies) by digesting 10 μl of the TC45 cDNA with 1 μl of BamHI (New England Biolabs), 1 μl of EcoRI (New England Biolabs), 3 μl 10×EcoRI buffer (New England Biolabs), 3 μl 10×BSA (New England Biolabs), and 12 μl distilled water for 3 hours at 37° C. Two microliters of the pENTR3C™ vector was digested with 0.5 μl of BamHI (New England Biolabs), 0.5 μl of EcoRI (New England Biolabs), 2 μl 10×EcoRI buffer (New England Biolabs), 2 μl 10×BSA (New England Biolabs), and 13 μl distilled water for 3 hours at 37° C., followed by an incubation of 30

minutes at 37° C. with calf intestinal phosphatase (New England Biolabs). Digested DNA was run on a 1% agarose gel, digested bands were excised and gel purified using a QIAGEN Gel Extraction kit (QIAGEN, Inc.). Four microliters of the TC45 cDNA was ligated into 2 μl of the pENTR3C™ vector overnight at 16° C. with 11 μl 10× Ligation Buffer (Invitrogen Life Technologies), 1 μl T4 DNA Ligase (4U/μl) (Invitrogen Life Technologies), and 2 μl distilled water. The construct was transformed into LIBRARY EFFICIENCY® DH5α™ cells. The FLAG® epitope-tagged TC45 construct was prepared by cloning the pENTR3C™ TC45 WT construct into the GWpCMVTag2 vector. The pENTR3C™ construct containing the TC45 polynucleotide was linearized by digesting the construct with Pvu I (New England Biolabs)) at 37° C. for 2 hours. The DNA was purified using a QIAGEN PCR Purification kit (QIAGEN, Inc.). Two microliters (150 ng/μl) of the GWpCMVTag2 vector were combined in a GATEWAY™ LR reaction with 3 μl linearized pENTR3C™ TC45 WT, 5 μl TE buffer, 4 μl Clonase™ Enzyme, and 4 μl LR reaction buffer (Invitrogen Life Technologies) overnight at room temperature. After addition of Proteinase K (Invitrogen Life Technologies) to the reaction for 10 minutes, LIBRARY EFFICIENCY® DH5α™ cells were transformed with the expression construct.

[0248] Cells (1BKO+HIR murine embryo fibroblasts) were co-transfected with an expression vector containing a nucleotide sequence encoding human TCPTP (SEQ ID NO:______) and siRNAs, hTCPTP1.4 (5'-guugucaugcugaaccgcatt-3' (SEQ ID NO:______)) (20 nM); hTCPTP1.5 (5'-gcccauaugaucacagucgtg-3' (SEQ ID NO:______)) (10 nM); hTCPTP1.6 (5'-ucgguuaaaugugcacaguac-3' (SEQ ID NO:______)) (10 nM); or hTCPTP1.7 (5'-ugacuauccucauagaguggg-3' (SEQ ID NO:______)) (20 nM). Additional human TCPTP specific siRNA polynucleotides were prepared; the sequences of each are as follows: hTCPTP1.1 (5'-agugagagaaucuggcucctt-3' (SEQ ID NO:______)); hTCPTP1.2 (5'-ggaagacuuaucuccugcctt-3' (SEQ ID NO:______)); and hTCPTP1.3 (5'-ggugaccgauguacaggactt-3' (SEQ ID NO:______)). The level of TCPTP expression was determined by immunoblotting with an anti-human TCPTP antibody. The level of expression of human TCPTP was not affected by siRNA hTCPTP1.7. Expression levels decreased more than 95% in the cells co-transfected with hTCPTP1.4; 80% in cells co-transfected with hTCPTP1.5; and greater than 90% in cells transfected with hTCPTP1.6.

[0249] Interference of Endogenous Expression of Human TCPTP by siRNA

[0250] 293-HEK HIR cells were transfected with either hTCPTP1.4 (SEQ ID NO:______) or rPTP1B1.2, a rat PTP1B sequence specific siRNA (5'-cggaugguggguggagguctt-3' (SEQ ID NO:______), which was included as a nonspecific siRNA control, at concentrations of 2, 5, 10, 20 and 50 nM. Endogenous expression of human TCPTP in the cells transfected with sequence specific hTCPTP1.4 decreased 90%.

[0251] Transient Transfection of Human PTP1B and Sequence Specific Hairpin Vectors

[0252] Effectiveness of a human PTP1B sequence-specific siRNA in the form of a hairpin insert was examined in a transient co-transfection assay. Cells (1BKO+HIR mouse fibroblasts) were transfected with a human PTP1B expression vector (see above) and co-transfected with hPTP1B hairpin vectors (1, 0.5, and 0.25 μg) according to the transfection method described above. The human PTP1B specific sequences were inserted in frame with a human U6 small nuclear RNA promoter into a vector, which was a gift from David Engelke (University of Michigan, Ann Arbor, Mich.) (see also Paul et al., *Nat. Biotechnol.* 20:446-48 (2002)). The sequences of each strand inserted into the hairpin vectors are as follows.

```
hPTP1B H1.2-HP4
5'-tttGCCCAAAGGAGTTACATTCGTAAGAATGTAACTCCTTTGGGCttttt-3'       (SEQ ID NO:___)

3' GGGTTTCCTCAATGTAAGCATTCTTACATTGAGGAAACCCGaaaaagatc-5'       (SEQ ID NO:___)

hPTP1B H1.2-HP9
5'-tttGCCCAAAGGAGTTACATTCCCTGGGTAAGAATGTAACTCCTTTGGGCttttt-3'  (SEQ ID NO:___)

3' GGGTTTCCTCAATGTAAGGGACCCATTCTTACATTGAGGAAACCCGaaaaagatc-5'  (SEQ ID NO:___)
```

[0253] Twenty-four hours after the cells were transfected, cell lysates were prepared and expression of human PTP1B was determined by immunoblotting with an antihuman PTP1B antibody (see above). Cell lysates were also immunoblotted with an antibody specific for human insulin receptor beta chain (IRβ) (Cat. No. C-19, Santa Cruz Biotechnology). The results are presented in **FIG. 19**.

[0254] Hairpin vectors are also prepared that contain sequences specific for murine PTP1B. The following sequences of each strand are inserted into a hairpin vector.

```
mPTP1BM1.1-HP4
5'-tttGAAGCCCAGAGGAGCTATAAGAATATAGCTCCTCTGGGCTTCttttt-3'       (SEQ ID NO:___)

3' TTCGGGTCTCCTCGATATTCTTATATCGAGGAGACCCGAAGaaaaagatc-5'       (SEQ ID NO:___)

mPTP1BM1.1-HP9
5'-tttGAAGCCCAGAGGAGCTATAGGGTGAGAATATAGCTCCTCTGGGCTTCttttt-3'  (SEQ ID NO:___)

3' TTCGGGTCTCCTCGATATCCCACTCTTATATCGAGGAGACCCGAAGaaaaagatc-5'  (SEQ ID NO:___)
```

EXAMPLE 6

Regulatory Role of TCPTP in Insulin Signaling

[0255] The protein tyrosine phosphatase TC-PTP exists in two alternatively spliced forms, TC45 and TC48, that share the same catalytic domain but differ at their extreme carboxy-termini (Mosinger et al., *Proc. Natl. Acad. Sci. USA* 89:499-503 (1992)). Insulin-induced oxidation and inactivation of TC45 suggested that it functions as a negative regulator of insulin signaling (see U.S. Ser. No. 10/366,547). This Example examines the regulatory role of TC45 in insulin signaling by inhibiting expression of the PTP by RNAi.

[0256] The specific siRNA duplexes were designed by first scanning through the open reading frame of TC45 mRNA and selecting sequences of 5'AA(N_{19})3' (N=any nucleotide) for further characterization. The following 2 oligonucleotides were chosen: 5'-AACAGAUACA-GAGAUGUAAGC-3' (TCPTP1) (SEQ ID NO:______) and 5'-AAGCCCA UAUGAUC ACAGUCG-3' (TCPTP2) (SEQ ID NO:______). These sequences were submitted to a BLAST search against human, rat, and mouse genome databases to ensure specificity for TC-PTP. The 21-nt siRNA duplexes were obtained in a deprotected and desalted form (Dharmacon Research). Rat-1 fibroblasts (Fischer rat fibroblast 3T3 like cell line) and HepG2 (human hepatocellular carcinoma) cells (American Type Culture Collection (ATCC), Manasass, Va.) were transfected with each siRNA at 100 nM. Both siRNA oligonucleotides suppressed expression of endogenous TC45 in the transfected HepG2 cells and Rat-1 fibroblasts, with TCPTP1 being more efficient.

[0257] Rat-1 (fibroblasts) and HepG2 (human hepatocellular carcinoma) cells were routinely maintained in DMEM supplemented with 10% FBS, 1% glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin. For stimulation with insulin, cells were plated in media containing 10% FBS for 48 hours, then serum-starved for 16 hours before treatment. For transient transfection, cells were plated in DMEM supplemented with 10% FBS for 16 hours, then in Opti-MEM (Invitrogen) without serum, after which the plasmid (5 μg/dish for Rat-1,30 μg/dish for HepG2) was introduced by LipofectAMINE and PLUS reagents (Invitrogen), according to the manufacture's recommendations. The transfection efficiency was routinely 40%. For RNAi experiments, cells were plated as above and the TCPTP siRNA duplexes were introduced by Oligofectamine (Invitrogen) according to the guidelines provided by Dharmacon Research Inc.

[0258] The potential regulatory role of TC45 in insulin signaling was investigated by examining the phosphorylation status of PKB/Akt, which is a critical effector in the P13 kinase pathway that mediates various intracellular responses to insulin, following ablation of the PTP by RNAi. The human hepatoma cell line HepG2 has been used extensively as a model to study insulin signaling (see Huang et al., *J. Biol. Chem.* 277:18151-60 (2002); Haj et al., *Science* 295 1708-11 (2002)). Serum-deprived Rat-I and HepG2 cells were exposed to 10 or 50 nM insulin for 5 min and lysed. The insulin receptor (IR) was immunoprecipitated from 500 μg of cell lysate with anti-IR-β antibody 29B4 (Santa Cruz Biotechnology), then immunoblotted with anti-phosphotyrosine, anti-pYpY$^{1162/1162}$-IR-β (Biosource International, Camarillo, Calif.) and anti-IR-β (C-19) (Santa Cruz Biotechnology) antibodies. HepG2 cells expressed higher levels of IR-β than Rat-1 cells as shown in **FIG. 20**A and displayed a robust response to insulin stimulation, as shown by the overall tyrosine phosphorylation level of IR-β and autophosphorylation of the activation loop tyrosines 1162 and 1163 (see **FIG. 20A**).

[0259] For the RNAi experiment, HepG2 cells were untransfected (control) or transfected (+siRNA) with 100 nM siRNA TCPTP1 oligonucleotide. Two days after transfection, cells were serum-starved for 16 hours and then stimulated with 10 nM insulin for 0, 1, 2, 5, 10, and 20 minutes. Total lysates (30 μg) were immunoblotted with anti-phospho-PKB/Akt (Cell Signaling Technology, Beverly, Mass.); anti-PKB/Akt (Cell Signaling Technology); anti-TC45 (1910H (Lorenzen et al., *J. Cell. Biol.* 131:631-43 (1995))); and anti-PTP1B (FG6 (LaMontagne et al., *Mol. Cell. Biol.* 18:2965-75 (1998))) antibodies. The results presented in **FIG. 20B** indicate that depletion of TC45 enhanced both the intensity and duration of the signaling response. **FIG. 20C** illustrates a densitometric analysis of the gel image to show the ratio of phosphorylated PKB/Akt relative to total PKB/Akt. Similar results were observed in three independent experiments.

[0260] The role of TC45 in insulin signaling was further investigated by preparing a TC45 substrate trapping mutant. Substitution of an alanine residue for the invariant aspartate, which functions as a general acid in catalysis, into the vector expressing TC45 and into a vector expressing PTP1B was performed by standard site-directed mutagenesis protocols. HepG2 cells overexpressing wild type (WT) or trapping mutant (DA) forms of PTP1B and TC45 were either left untreated (−INS) or stimulated with 10 nM insulin for 5 min (+INS), then lysed in trapping buffer (20 mM Tris (pH 7.4), 1% NP-40, 150 mM NaCl, 10% glycerol, 10 mM IAA and 25 μg/ml each of aprotinin and leupeptin). Aliquots (1 mg) of cell lysate were incubated with anti-PTP1B antibody (FG6) or anti-TC45 antibody (CF4). The immunocomplexes were washed with lysis buffer, subjected to SDS-PAGE then immunoblotted with anti-IR-β (C-19) antibody. An aliquot of lysate (30 μg) was immunoblotted with anti-PTP1B antibody (FG6) or anti-TC-PTP antibody (CF4) to verify PTP expression. The data are shown in **FIG. 21A** and are representative of three independent experiments. These data suggest that TC45 recognizes IR-β as a substrate.

[0261] Serum starved, untransfected (control) or TC45 siRNA (100 nM) transfected (+siRNA) HepG2 cells were stimulated with 10 nM insulin for 0, 1, 2, 5, 10, and 20 minutes. The insulin receptor was immunoprecipitated from 750 μg of cell lysate with anti-IR-β antibody 29B4 and immunoblotted with anti-phosphotyrosine (G104), anti-pY^{972}-β (Biosource), anti-pYpY$^{1162/1163}$-IR-β, and anti-IR-β (C-19) antibodies as shown in **FIG. 21B**. **FIG. 21C** illustrates densitometric analyses of the gel image to show the ratio of phosphorylated IR-β relative to total IR-β for total phosphotyrosine (upper panel), phosphorylation of Tyr 972 (middle panel), and phosphorylation of the activation loop tyrosines 1162 and 1163 (lower panel). Similar results were observed in two independent experiments.

EXAMPLE 7

Effect of siRNAs Specific for PTP1B and TCPTP on Insulin Receptor Tyrosine Phosphorylation

[0262] This example illustrates the effect of RNAi on the function of components in a cell signaling pathway. The role of PTP1B in the down regulation of insulin signaling has been illustrated by data derived from a variety of approaches (Cheng et al., *Eur. J. Biochem.* 269:1050-59 (2002)), including the phenotype of the PTP1B knockout mouse (Elchebly et al., *Science* 283:1544-48 (1999); Klaman et al., *Mol. Cell Biol* 20:5479-89 (2000); see also U.S. patent application Ser. No. 10/366,547).

[0263] The effect of human PTP1B siRNA and of human TCPTP siRNA on the level of phosphorylation of IR-β was evaluated by ELISA. 292-HEK HIR cells were transfected with 0, 0.5, 3, or 10 nM siRNAs. The siRNA polynucleotides transfected into the cells included hPTP1B1.2 (SEQ ID NO:______), hPTP1B1.3 (SEQ ID NO:______), mPTP1B1.1 (SEQ ID NO:______), rPTP1B1.2 (SEQ ID NO:______), hTCPTP1.4 (SEQ ID NO:______), and the combination of hPTP1B1.3 and hTCPTP1.4. Seventy-two hours after transfection, cells were exposed to insulin for 7 minutes at concentrations of 0, 25, 50, 75, and 100 nM. Cell lysates were prepared as described in Example 1, and total cell protein was quantified by the Bio-Rad Protein Assay performed according to the manufacturer's instructions (BioRad, Hercules, Calif.). An ELISA was performed as follows. Dynex Immulon HB4X plates were coated with anti-insulin receptor antibody Ab-1 (1 mg/ml; NeoMarkers, Inc., Fremont, Calif.) that was diluted 1:1000 in CMF (calcium magnesium free)-PBS containing 5 μg/ml fatty acid free BSA (faf-BSA). The plates were incubated at 4° C. for at least four hours. The antibody solution was removed by aspiration, followed by the addition of 300 μl of 3% faf-BSA+CMF-PBS. The plates were incubated for 1 hr with agitation on a vortex platform shaker (setting #5) at room temperature. After aspirating the 3% faf-BSA+CMF-PBS solution, approximately 10-20 μg of lysate were added to the wells and incubated at room temperature for one hour. Plates were washed three times with TBST (20 mM Tris-HCl, pH 7.5 150 mM NaCl; 0.05% Tween 20). An anti-insulin receptor phosphotyrosine specific antibody (pTyr 1162/63, Biosource International, Camarillo, Calif., Catalog #44-804) was diluted 1:2000 in TBST and added to the plates for one hour at room temperature. The plates were washed three times with TBST. HRP-conjugated anti-rabbit antibody (Amersham Biosciences, catalog #NA934V) (1:2000 in TBST) was then added to the wells and incubated at room temperature for one hour. The plates were washed three times with TBST and once with deionized, sterile water. TMB solution (Sigma Aldrich) (100 μl per well) was added and developed until a modest color change (10-30 minutes depending on cell type and insulin response). The reaction was stopped with 100 μl of 1.8 N H_2SO_4 and then mixed. The optical density of each well was measured at 450 nM in a Spectramax plate reader (Molecular Devices Corp., Sunnyvale, Calif.). The data are presented in **FIG. 22**. The level of expression of PTP1B in each cell lysates was determined by immunoblot as described above. PTP1B polypeptide was detected using an anti-human PTP-1B antibody (PHO2, Oncogene Research Products™, Inc.). The amount of PTP1B expressed in cells transfected with varying concentrations of either siRNA was quantified by densitometric analysis of the immunoblot. The level of expression of human PTP1B is presented as a percent of the level of expression in cells that were not transfected with hPTP1B1.3 siRNA (i.e., the level of expression in untransfected cells equals 100%) (see tables in **FIG. 22**).

[0264] In a second experiment, 292-HEK HIR cells were transfected with 0, 0.5, 3, or 10 nM siRNAs. The siRNA polynucleotides transfected into the cells included hPTP1B1.2 (SEQ ID NO:______), hPTP1B1.3 (SEQ ID NO:______), mPTP1B1.1 (SEQ ID NO:______), hTCPTP1.4 (SEQ ID NO:______), and rPTP1B1.2 (SEQ ID NO:______). Seventy-two hours after transfection, cells were exposed to insulin for 7 minutes at concentrations of 0, 5, 10, 20, 50, and 100 nM. Cell lysates were prepared and total cell protein was quantified as described above. An ELISA was performed as described above. Cell lysates were coated onto 96-well plates, blocked, and probed with an anti-pYpY$^{1162/1163}$-IR-β antibody. Binding was detected using an enzyme conjugated secondary reagent. As shown in FIGS. **23** and **24**, respectively, increased phosphorylation of the insulin receptor was observed in cells transfected with hPTP1B1.3 and with hTCPTP1.4.

[0265] The percent decrease in the level of PTP1B expression was compared with the level of phosphorylation of the insulin receptor. In three separate experements, 292-HEK HIR cells were transfected with 0, 0.5, 3, or 10 nM hPTP1B1.3 siRNA and then exposed to insulin for 7 minutes at concentrations of 0, 5, 10, 20, 50, and 100 nM. An ELISA and immunoblot of cell lysates were performed as described above. The effect of hPTP1B1.3 siRNA on the phosphorylation state of the insulin receptor is summarized in **FIG. 25**. Each data point represents the average optical density measured in duplicate wells.

EXAMPLE 8

Identification of Oncology Targets and Decreased Expression of the Targets by Specific siRNAs

[0266] This Example describes validation of DSP-3 as a target for oncology therapeutics. The Example also describes identification of siRNA polynucleotides that effectively interfere with expression of known chemotherapeutic target polypeptides.

[0267] Expression of DSP-3 polypeptide was evaluated in several cancer cell lines transfected with sequence specific DSP-3 siRNA polynucleotides and nonspecific siRNA polynucleotides. Cell lines included HeLa, HS578T; MDA-MB-231; MDA-MB-435 (breast cancer cell line that is ER^-, Her^{2+}, $EGFR^+$, $p53^{mut}$, and invasive); MCF7 (breast cancer cell line that is ER^+, $Her2^{low}$, $EGFR^{low}$, $p53^{WT}$, and non-invasive); T47D (breast cancer cell line that is ER^+, $Her2^-$, $EGFR^-$, $p53^{mut}$, and non-invasive); HCT-116 ($p53^{WT}$); and HT-29 ($p53^{mut}$). Cells were transfected with 10 nM DSP3.1 (SEQ ID NO:______), DSP3.4 (5'-ggugacacauauucugucutt-3', (SEQ ID NO:______)), or Scr.2 (SEQ ID NO:______) (scrambled, a non-specific siRNA sequence not found in a human genome database), and then cell lysates were prepared and evaluated for expression of DSP-3 and inhibition of expression by specific siRNAs, as described in Example 1. Transfection efficiency of some cell lines with siRNA, for example, MC7 and T47D, was improved by using Lipofectamine™ 2000 according to manufacturer's recommen-

dations (Invitrogen Life Technologies) rather than Oligofectamine™ (Invitrogen Life Technologies) for the transfection procedure. The level of expression of DSP-3 polypeptide in the presence of specific siRNA 4compared with the non-specific siRNA control was significantly decreased in MCF7, T47D, MD-MB-435, HCT-116, and HT-29 cells.

[0268] Interference with expression of known chemotherapeutic targets by RNAi was examined, and siRNA polynucleotides that effectively interfere with expression of the targets were identified. Targets included dihydrofolate reductase (DHFR) (GenBank Accession No. NM_000791) (SEQ ID NOs:______ and ______); thymidylate synthetase (GenBank Accession No. NM_001071) (SEQ ID NOs:______ and ______); and topoisomerase I (GenBank Accession No. J03250) (SEQ ID NOs:______ and ______). The siRNA polynucleotides were designed according to methods described in Examples 1 and 2 and were manufactured by Dharmacon. Each siRNA was transfected into HeLa cells, and the effect of each on the endogenous expression of DHFR, thymidylate synthetase, and topoisomerase I was evaluated by immunoblotting of cell lysates as described in Example 1. The level of expression of the targets was determined by immunoblotting with an anti-DHFR monoclonal antibody (BD monoclonal antibody (diluted 1:250)); an anti-topoisomerase I antibody (Santa Cruz Biotechnology, Cat. No. sc-10783, diluted 1:200); and an anti-thymidylate synthetase antibody (Rockland sheep polyclonal antibody diluted 1:2000). The results are presented in Table 3.

TABLE 14

siRNA INTERFERENCE WITH ENDOGENOUS EXPRESSION OF DHFR, THYMIDYLATE SYNTHETASE, AND TOPOISOMERASE I

Target	siRNA Sequence (SEQ ID NO)	siRNA Name	Related SEQ ID NO:	Decrease in Expression
DHFR	5'-gaccugguucuccauuccutt-3'	DHFR.1		>90%
	5'-gcaguguauuugcuagguctt-3'	DHFR.3		>80%
	5'-gucagcgagcagguucucatt-3'	DHFR.4		>90%
Thymidylate Synthetase	5'-ccaaacguguguucuggaatt-3'	TYMS.1		>95%
	5'-ccaacccugacgacagaagtt-3'	TYMS.2		>90%
	5'-gccaggugacuuuauacactt-3'	TYMS.3		>95%
	5'-cccagaccuuucccaaagctt-3'	TYMS.4		>90%
Topoisomerase I	5'-gauagagccucuggacuutt-3'	TOP1.1		>90%
	5'-guccggcaugauaacaaggtt-3'	TOP1.2		>90%
	5'-ggagaaacagcggacacugtt-3'	TOP1.3		>80%
	5'-gcagcccgaggaugaucuutt-3'	TOP1.4		>80%

[0269] Interference of expression of another chemotherapeutic polypeptide target IKKgamma is performed according to the same procedures described above. The siRNA polynucleotides that are tested are IKK.1 (5'-gagucuccucuggggaagctt-3' (SEQ ID NO:______)); IKK.2 (5'-ggaguuccucaugugcaagtt-3' (SEQ ID NO:______)); IKK.3 (5'-ggccucugugaaagcccagtt-3' (SEQ ID NO:______)); and IKK.4 (5'-cacgcugcucuugauguggtt-3' (SEQ ID NO:______)).

[0270] The effect of RNAi silencing on expression of DHFR was compared with silencing of DSP-3, Cdc14a, and SHP-2 polypeptide expression in a HCT-116 cell proliferation assay. HCT-116 cells were transfected with 2.5 nM of the following siRNA oligonucleotides: scr.2 (SEQ ID NO:______); DSP3.1 (SEQ ID NO:______); DSP3.4 (SEQ ID NO:______); cdc14a.3 (SEQ ID NO:______); cdc14a.5 (SEQ ID NO:______); SHP2.1 (SEQ ID NO:______); SHP2.2 (SEQ ID NO:______); and DHFR.1 (SEQ ID NO:______). After 5 days, cell proliferation was evaluated by performing an MTT assay essentially as described in Example 4. The results are presented in **FIG. 26**. The optical density (OD) measured for each siRNA represents an average of six wells.

[0271] A cell proliferation assay was also performed using a different cell line, T47D, and the same siRNAs. The data are presented in **FIG. 27**. The effect of silencing on proliferation was confirmed by cell counting. The number of T47D cells transfected with the nonspecific control siRNA scr.2 was approximately 200×10^4. In T47D cells transfected with either DSP3.1 or DSP3.4 siRNA, the number of cells was approximately 75% of the negative control, and in the presence of DHFR.1, the number of cells was approximately 50% compared with cells transfected with the nonspecific control. Significantly decreased expression of DSP-3 and DHFR in cells transfected with the respective siRNAs was confirmed by immunoblot.

[0272] Silencing of DSP-3 in HCT-116 and T47D cells also induced proapoptotic signaling. HCT-116 cells and T47D cells were transfected with 10 nM of non-specific si RNA control scrb1.2 (SEQ ID NO:______) (identical sequence to scr.2 described above), DSP3.1, DSP3.4, or DHFR.1. Three days after transfection of HCT-116 cells and five days after transfection of T47D cells, PARP assays were performed as described in Example 4. The results are presented in **FIG. 28**.

EXAMPLE 9

Inhibition of MAP Kinase Kinase Expression by RNAi

[0273] This Example describes interference of expression of MAP kinase kinases that are involved in the JNK signal transduction pathway in cells transfected with sequence specific siRNA polynucleotides.

[0274] Transient co-transfection experiments were performed as described in Example 2. 293-HEK cells were co-transfected with an expression vector that contained a polynucleotide sequence (GenBank Accession No. L36870 (SEQ ID NO:______)) that encoded FLAG®-tagged human MKK4 polypeptide (GenBank Accession No. L36870 (SEQ ID NO:______)) or with an expression vector that contained a polynucleotide sequence (GenBank Accession No. AF013588 (SEQ ID NO:______)) that encoded FLAG®-tagged human MKK7 polypeptide (GenBank Accession No. AF013588 (SEQ ID NO:______)). The siRNA oligonucleotides were designed and prepared as described in Examples 1 and 2. The cells were transfected and the level of expression of each kinase was determined by immunoblotting with an anti-FLAG® monoclonal antibody as described in Example 2. The results are presented in Table 4.

TABLE 15

siRNA INTERFERENCE WITH MKK4 AND MKK7 EXPRESSION IN CO-TRANSFECTION ASSAYS

Target	siRNA Sequence (SEQ ID NO)	siRNA Name	Related SEQ ID NOs	Decrease in Expression
MKK4	5'-gugggcaaauaauggcagutt-3'	MKK4.1		80%
	5'-cugugaaagcacuaaaccatt-3'	MKK4.2		90%
	5'-ggagauccuccgcagcugatt-3'	MKK4.3		90%
	5'-gcucuuuauacuuuggccutt-3'	MKK4.4		80%
MKK7	5'-gcagacgggcuaccugacctt-3'	MKK7.1		10%
	5'-cacggacgucuucaucgcctt-3'	MKK7.2		10%
	5'-gaagcggaugcagggcccctt-3'	MKK7.3		10%
	5'-cugcaagacggacuuugagtt-3'	MKK7.4		10%

EXAMPLE 10

Inhibition of Human P53 Expression by RNAi

[0275] An hairpin vector is prepared that contains a polynucleotide insert comprising a sequence that is a portion of a polynucleotide that encodes human p53 as described in Example 5. This sequence may be incorporated into a hairpin vector and transfected into a cell line known to express p53 (see Example 5). The level of expression of p53 is then determined by methods well known in the art, such as immunoblotting using an anti-p53 antibody (see Example 5). The p53 sequence incorporated into a hairpin vector is as follows.

[0276] HP53-HP9

[0277] 5'-tttGACTCCAGTGGTMTCTACTTCM-GAGAGTAGATTACCACTGGAGTCttttt-3' (SEQ ID NO:______)

[0278] 3' tgaggtcaccattagatgaagttctct-catctaatggtgacctcagAAAAAGATC-5' (SEQ ID NO:______)

ADDITIONAL REFERENCES

[0279] Agami et al., *Cell* 102:55-66 (2000)

[0280] Bass, Brenda L., *Cell* 101:235:238 (2000)

[0281] Brummelkamp et al., *Science* 296:550-53 (2002)

[0282] Carthew, Richard W., *Current Opinion in Cell Biology* 13:244-248 (2001)

[0283] Clemens et al., *Proc. Natl. Acad. Sci. USA* 97:6499-6503 (2000)

[0284] Elbashir et al., *Genes & Development* 15:188-200 (2001)

[0285] Elbashir, et al., *Nature* 411:494-498 (2001)

[0286] Fire et al., *Nature* 391:806-11 (1993)

[0287] Flint et al., *Proc. Natl. Acad. Sci. USA* 94:1680-1685 (1997)

[0288] Fukada et al., *J. Biol. Chem.* 276:25512-25519 (2001)

[0289] Harborth et al., *J. Cell Sci.* 114:4557-4565 (2001)

[0290] Hutvagner et al., *Curr. Opin. Gen. & Dev.* 12:225-232 (2002)

[0291] Kisielow et al., *Biochem. J.* 363:1-5 (2002)

[0292] Paddison et al., *Genes & Development* 16:948-958 (2002)

[0293] Salmeen et al., *Moleular Cell* 6:1401-1412 (2000)

[0294] Scadden et al., *EMBO Reports* 2:1107-1111 (2001)

[0295] Sharp, Phillip A., *Genes & Development* 13:139-141 (1999)

[0296] Sharp, Phillip A., *Genes & Development* 15:485-490 (2001)

[0297] Shen et al., *Proc. Natl. Acad. Sci. USA* 24:13613-13618 (2001)

[0298] Sui et al., *Proc. Natl. Acad. Sci. USA* 99:5515-5520 (2002)

[0299] Tonks et al, *Curr. Opin. Cell Biol.* 13:182-195 (2001)

[0300] Tuschl, Thomas, *Chembiochem.* 2:239-245 (2001)

[0301] Ui-Tei et al., *FEBS Letters* 479:79-82 (2000)

[0302] Wen et al., *Proc. Natl. Acad. Sci.* 98:4622-4627 (2001)

[0303] Zamore et al., *Cell* 101:25-33 (2000)

[0304] EP1 152 056

[0305] U.S. Pat. No. 2001/0029617

[0306] U.S. Pat. No. 2002/0007051

[0307] U.S. Pat. No. 6,326,193

[0308] U.S. Pat. No. 6,342,595

[0309] U.S. Pat. No. 6,506,559

[0310] WO 01/29058

[0311] WO 01/34815

[0312] WO 01/42443

[0313] WO 01/68836

[0314] WO 01/75164

[0315] WO 01/92513

[0316] WO 01/96584

[0317] WO 99/32619

[0318] From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for the purpose of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the present invention is not limited except as by the appended claims.

```
                              SEQUENCE LISTING


<160> NUMBER OF SEQ ID NOS: 842

<210> SEQ ID NO 1
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Unknown
<220> FEATURE:
<223> OTHER INFORMATION: Unique signature sequence motif contained
      within the conserved domain of  the PTP family of enzymes
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: 2, 3, 4, 5, 6
<223> OTHER INFORMATION: Xaa = Any Amino Acid

<400> SEQUENCE: 1

Cys Xaa Xaa Xaa Xaa Xaa Arg
1               5


<210> SEQ ID NO 2
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: UNKNOWN
<220> FEATURE:
<223> OTHER INFORMATION: 11 amino acid conserved sequence conatining the
      signature sequence motif for the majority of PTPs.
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: 1
<223> OTHER INFORMATION: Xaa = Ile or Val
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: 4,7,8
<223> OTHER INFORMATION: Xaa = any amino acid
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: 10
<223> OTHER INFORMATION: Xaa = Ser or Thr

<400> SEQUENCE: 2

Xaa His Cys Xaa Ala Gly Xaa Xaa Arg Xaa Gly
1               5                   10


<210> SEQ ID NO 3
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP3.1

<400> SEQUENCE: 3

cgauagugcc aggccuaugt t                                                21
```

-continued

<210> SEQ ID NO 4
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP3.1

<400> SEQUENCE: 4

cgauagugcc aggccuaug 19

<210> SEQ ID NO 5
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP3.1

<400> SEQUENCE: 5

cauaggccug gcacuaucg 19

<210> SEQ ID NO 6
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP3.1
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 6

cgauagugcc aggccuaugn n 21

<210> SEQ ID NO 7
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP3.1
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 7

nncauaggcc uggcacuauc g 21

<210> SEQ ID NO 8
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP3.2

<400> SEQUENCE: 8

gcaugagguc caucaguaut t 21

<210> SEQ ID NO 9
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP3.2

-continued

<400> SEQUENCE: 9

gcaugagguc caucaguau 19

<210> SEQ ID NO 10
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP3.2

<400> SEQUENCE: 10

auacugaugg accucaugc 19

<210> SEQ ID NO 11
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP3.2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 11

gcaugagguc caucaguaun n 21

<210> SEQ ID NO 12
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP3.2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 12

nnauacugau ggaccucaug c 21

<210> SEQ ID NO 13
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP3.3

<400> SEQUENCE: 13

cgauacugcc aggcccaugt t 21

<210> SEQ ID NO 14
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP3.3

<400> SEQUENCE: 14

cgauacugcc aggcccaug 19

<210> SEQ ID NO 15
<211> LENGTH: 19

-continued

<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP3.3

<400> SEQUENCE: 15

caugggccug gcaguaucg 19

<210> SEQ ID NO 16
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP3.3
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 16

cgauacugcc aggcccaugn n 21

<210> SEQ ID NO 17
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP3.3
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 17

nncaugggcc uggcaguauc g 21

<210> SEQ ID NO 18
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - MKP.1

<400> SEQUENCE: 18

auccugcccu uucuguacct t 21

<210> SEQ ID NO 19
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - MKP.1

<400> SEQUENCE: 19

auccugcccu uucuguacc 19

<210> SEQ ID NO 20
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - MKP.1

<400> SEQUENCE: 20

gguacagaaa gggcaggau 19

-continued

```
<210> SEQ ID NO 21
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - MKP.1
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 21

auccugcccu uucuguaccn n                                              21


<210> SEQ ID NO 22
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - MKP.1
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 22

nngguacaga aagggcagga u                                              21


<210> SEQ ID NO 23
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - MKP.2

<400> SEQUENCE: 23

gcagaggcaa agcaucauct t                                              21


<210> SEQ ID NO 24
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - MKP.2

<400> SEQUENCE: 24

gcagaggcaa agcaucauc                                                 19


<210> SEQ ID NO 25
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - MKP.2

<400> SEQUENCE: 25

gaugaugcuu ugccucugc                                                 19


<210> SEQ ID NO 26
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - MKP.2
```

-continued

<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 26

gcagaggcaa agcaucaucn n 21

<210> SEQ ID NO 27
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - MKP.2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 27

nngaugaugc uuugccucug c 21

<210> SEQ ID NO 28
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc14a.1

<400> SEQUENCE: 28

caucgugcga agguuccugt t 21

<210> SEQ ID NO 29
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc14a.1

<400> SEQUENCE: 29

caucgugcga agguuccug 19

<210> SEQ ID NO 30
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc14a.1

<400> SEQUENCE: 30

caggaaccuu cgcacgaug 19

<210> SEQ ID NO 31
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc14a.1
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 31

caucgugcga agguuccugn n 21

-continued

<210> SEQ ID NO 32
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc14a.1
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 32

nncaggaacc uucgcacgau g 21

<210> SEQ ID NO 33
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - CD45.2

<400> SEQUENCE: 33

gccgagaaca aaguggaugt t 21

<210> SEQ ID NO 34
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - CD45.2

<400> SEQUENCE: 34

gccgagaaca aaguggaug 19

<210> SEQ ID NO 35
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - CD45.2

<400> SEQUENCE: 35

cauccacuuu guucucggc 19

<210> SEQ ID NO 36
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - CD45.2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 36

gccgagaaca aaguggaugn n 21

<210> SEQ ID NO 37
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - CD45.2

-continued

<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 37

nncauccacu uuguucucgg c 21

<210> SEQ ID NO 38
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: FLAG sequence

<400> SEQUENCE: 38

Asp Tyr Lys Asp Asp Asp Asp Lys
1 5

<210> SEQ ID NO 39
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP11.2

<400> SEQUENCE: 39

cuggcaccau gcuggccugt t 21

<210> SEQ ID NO 40
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP11.2

<400> SEQUENCE: 40

cuggcaccau gcuggccug 19

<210> SEQ ID NO 41
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP11.2

<400> SEQUENCE: 41

caggccagca uggugccag 19

<210> SEQ ID NO 42
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP11.2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 42

cuggcaccau gcuggccugn n 21

<210> SEQ ID NO 43

-continued

<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP11.2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 43

nncaggccag cauggugcca g 21

<210> SEQ ID NO 44
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP11.4

<400> SEQUENCE: 44

agcagucuuc caguucuact t 21

<210> SEQ ID NO 45
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP11.4

<400> SEQUENCE: 45

agcagucuuc caguucuac 19

<210> SEQ ID NO 46
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP11.4

<400> SEQUENCE: 46

guagaacugg aagacugcu 19

<210> SEQ ID NO 47
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP11.4
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 47

agcagucuuc caguucuacn n 21

<210> SEQ ID NO 48
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP11.4
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2

-continued

<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 48

nnguagaacu ggaagacugc u 21

<210> SEQ ID NO 49
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP18.2

<400> SEQUENCE: 49

cugccuugug cacugcuuut t 21

<210> SEQ ID NO 50
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP18.2

<400> SEQUENCE: 50

cugccuugug cacugcuuu 19

<210> SEQ ID NO 51
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP18.2

<400> SEQUENCE: 51

aaagcagugc acaaggcag 19

<210> SEQ ID NO 52
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP18.2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 52

cugccuugug cacugcuuun n 21

<210> SEQ ID NO 53
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP18.2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 53

nnaaagcagu gcacaaggca g 21

<210> SEQ ID NO 54

-continued

```
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP18.4

<400> SEQUENCE: 54

gaguuuggcu gggccaguut t                                              21


<210> SEQ ID NO 55
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP18.4

<400> SEQUENCE: 55

gaguuuggcu gggccaguu                                                 19


<210> SEQ ID NO 56
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP18.4

<400> SEQUENCE: 56

aacuggccca gccaaacuc                                                 19


<210> SEQ ID NO 57
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP18.4
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 57

gaguuuggcu gggccaguun n                                              21


<210> SEQ ID NO 58
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP18.4
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 58

nnaacuggcc cagccaaacu c                                              21


<210> SEQ ID NO 59
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP13.1

<400> SEQUENCE: 59
```

-continued

```
cuugcgggaa uucaaggaat t                                          21


<210> SEQ ID NO 60
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP13.1

<400> SEQUENCE: 60

cuugcgggaa uucaaggaa                                             19


<210> SEQ ID NO 61
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP13.1

<400> SEQUENCE: 61

uuccuugaau ucccgcaag                                             19


<210> SEQ ID NO 62
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP13.1
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 62

cuugcgggaa uucaaggaan n                                          21


<210> SEQ ID NO 63
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP13.1
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 63

nnuuccuuga auucccgcaa g                                          21


<210> SEQ ID NO 64
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP13.2

<400> SEQUENCE: 64

ccgaggggua cgguauauct t                                          21


<210> SEQ ID NO 65
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
```

-continued

```
<223> OTHER INFORMATION: Small interfering RNA - DSP13.2

<400> SEQUENCE: 65

ccgaggggua cgguauauc                                                19


<210> SEQ ID NO 66
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP13.2

<400> SEQUENCE: 66

gauauaccgu accccucgg                                                19


<210> SEQ ID NO 67
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP13.2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 67

ccgaggggua cgguauaucn n                                             21


<210> SEQ ID NO 68
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP13.2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 68

nngauauacc guaccccucg g                                             21


<210> SEQ ID NO 69
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP13.3

<400> SEQUENCE: 69

caucaggcug gcuguaagat t                                             21


<210> SEQ ID NO 70
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP13.3

<400> SEQUENCE: 70

caucaggcug gcuguaaga                                                19


<210> SEQ ID NO 71
```

-continued

<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP13.3

<400> SEQUENCE: 71

ucuuacagcc agccugaug 19

<210> SEQ ID NO 72
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP13.3
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 72

caucaggcug gcuguaagan n 21

<210> SEQ ID NO 73
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP13.3
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 73

nnucuuacag ccagccugau g 21

<210> SEQ ID NO 74
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP13.4

<400> SEQUENCE: 74

cauggaucua aaugccuugt t 21

<210> SEQ ID NO 75
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP13.4

<400> SEQUENCE: 75

cauggaucua aaugccuug 19

<210> SEQ ID NO 76
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP13.4

<400> SEQUENCE: 76

-continued

caaggcauuu agauccaug 19

<210> SEQ ID NO 77
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP13.4
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 77

cauggaucua aaugccuugn n 21

<210> SEQ ID NO 78
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP13.4
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 78

nncaaggcau uuagauccau g 21

<210> SEQ ID NO 79
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP14.1

<400> SEQUENCE: 79

gugaagacaa gccucaagat t 21

<210> SEQ ID NO 80
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP14.1

<400> SEQUENCE: 80

gugaagacaa gccucaaga 19

<210> SEQ ID NO 81
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP14.1

<400> SEQUENCE: 81

ucuugaggcu ugucuucac 19

<210> SEQ ID NO 82
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

-continued

<223> OTHER INFORMATION: Small interfering RNA - DSP14.1
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 82

gugaagacaa gccucaagan n 21

<210> SEQ ID NO 83
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP14.1
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 83

nnucuugagg cuugucuuca c 21

<210> SEQ ID NO 84
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP14.2

<400> SEQUENCE: 84

gcucuacauu ggcgaugagt t 21

<210> SEQ ID NO 85
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP14.2

<400> SEQUENCE: 85

gcucuacauu ggcgaugag 19

<210> SEQ ID NO 86
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP14.2

<400> SEQUENCE: 86

cucaucgcca auguagagc 19

<210> SEQ ID NO 87
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP14.2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 87

-continued

gcucuacauu ggcgaugagn n 21

<210> SEQ ID NO 88
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP14.2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 88

nncucaucgc caauguagag c 21

<210> SEQ ID NO 89
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP14.3

<400> SEQUENCE: 89

gcgacgacca caguaagaut t 21

<210> SEQ ID NO 90
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP14.3

<400> SEQUENCE: 90

gcgacgacca caguaagau 19

<210> SEQ ID NO 91
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP14.3

<400> SEQUENCE: 91

aucuuacugu ggucgucgc 19

<210> SEQ ID NO 92
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP14.3
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 92

gcgacgacca caguaagaun n 21

<210> SEQ ID NO 93
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

-continued

<223> OTHER INFORMATION: Small interfering RNA - DSP14.3
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 93

nnaucuuacu guggucgucg c 21

<210> SEQ ID NO 94
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP14.4

<400> SEQUENCE: 94

ggacaugacc cugguggact t 21

<210> SEQ ID NO 95
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP14.4

<400> SEQUENCE: 95

ggacaugacc cugguggac 19

<210> SEQ ID NO 96
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP14.4

<400> SEQUENCE: 96

guccaccagg gucaugucc 19

<210> SEQ ID NO 97
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP14.4
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 97

ggacaugacc cugguggacn n 21

<210> SEQ ID NO 98
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - DSP14.4
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 98

-continued

```
nnguccacca gggucauguc c                                              21


<210> SEQ ID NO 99
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - SHP2.1

<400> SEQUENCE: 99

gauucagaac acuggugaut t                                              21


<210> SEQ ID NO 100
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - SHP2.1

<400> SEQUENCE: 100

gauucagaac acuggugau                                                 19


<210> SEQ ID NO 101
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - SHP2.1

<400> SEQUENCE: 101

aucaccagug uucugaauc                                                 19


<210> SEQ ID NO 102
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - SHP2.1
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 102

gauucagaac acuggugaun n                                              21


<210> SEQ ID NO 103
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - SHP2.1
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 103

nnaucaccag uguucugaau c                                              21


<210> SEQ ID NO 104
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
```

-continued

<223> OTHER INFORMATION: Small interfering RNA - SHP2.2

<400> SEQUENCE: 104

gaauauggcg ucaugcgugt t 21

<210> SEQ ID NO 105
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - SHP2.2

<400> SEQUENCE: 105

gaauauggcg ucaugcgug 19

<210> SEQ ID NO 106
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - SHP2.2

<400> SEQUENCE: 106

cacgcaugac gccauauuc 19

<210> SEQ ID NO 107
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - SHP2.2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 107

gaauauggcg ucaugcgugn n 21

<210> SEQ ID NO 108
<211> LENGTH: 21
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - SHP2.2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 108

nncacgcaug acgccauauu c 21

<210> SEQ ID NO 109
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - SHP2.3

<400> SEQUENCE: 109

cggucuggca auaccacuut t 21

<210> SEQ ID NO 110

-continued

<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - SHP2.3

<400> SEQUENCE: 110

cggucuggca auaccacuu 19

<210> SEQ ID NO 111
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - SHP2.3

<400> SEQUENCE: 111

aagugguauu gccagaccg 19

<210> SEQ ID NO 112
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - SHP2.3
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 112

cggucuggca auaccacuun n 21

<210> SEQ ID NO 113
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - SHP2.3
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 113

nnaaguggua uugccagacc g 21

<210> SEQ ID NO 114
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - SHP2.4

<400> SEQUENCE: 114

ugacggcaag ucuaaagugt t 21

<210> SEQ ID NO 115
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - SHP2.4

<400> SEQUENCE: 115

-continued

```
ugacggcaag ucuaaagug                                                  19


<210> SEQ ID NO 116
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - SHP2.4

<400> SEQUENCE: 116

cacuuuagac uugccguca                                                  19


<210> SEQ ID NO 117
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - SHP2.4
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 117

ugacggcaag ucuaaagugn n                                               21


<210> SEQ ID NO 118
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - SHP2.4
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 118

nncacuuuag acuugccguc a                                               21


<210> SEQ ID NO 119
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - KAP.1

<400> SEQUENCE: 119

gagccuauug aagaugaact t                                               21


<210> SEQ ID NO 120
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - KAP.1

<400> SEQUENCE: 120

gagccuauug aagaugaac                                                  19


<210> SEQ ID NO 121
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
```

-continued

<223> OTHER INFORMATION: Small interfering RNA - KAP.1

<400> SEQUENCE: 121

guucaucuuc aauaggcuc 19

<210> SEQ ID NO 122
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - KAP.1
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 122

gagccuauug aagaugaacn n 21

<210> SEQ ID NO 123
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - KAP.1
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 123

nnguucaucu ucaauaggcu c 21

<210> SEQ ID NO 124
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - KAP.2

<400> SEQUENCE: 124

gagcuguggu auacaagact t 21

<210> SEQ ID NO 125
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - KAP.2

<400> SEQUENCE: 125

gagcuguggu auacaagac 19

<210> SEQ ID NO 126
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - KAP.2

<400> SEQUENCE: 126

gucuuguaua ccacagcuc 19

<210> SEQ ID NO 127

-continued

<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - KAP.2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 127

gagcuguggu auacaagacn n 21

<210> SEQ ID NO 128
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - KAP.2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 128

nngucuugua uaccacagcu c 21

<210> SEQ ID NO 129
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - KAP.3

<400> SEQUENCE: 129

gagcuuacaa ccugccuuat t 21

<210> SEQ ID NO 130
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - KAP.3

<400> SEQUENCE: 130

gagcuuacaa ccugccuua 19

<210> SEQ ID NO 131
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - KAP.3

<400> SEQUENCE: 131

uaaggcaggu uguaagcuc 19

<210> SEQ ID NO 132
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - KAP.3
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21

-continued

<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 132

gagcuuacaa ccugccuuan n 21

<210> SEQ ID NO 133
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - KAP.3
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 133

nnuaaggcag guuguaagcu c 21

<210> SEQ ID NO 134
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - KAP.4

<400> SEQUENCE: 134

uacacugcua uggaggacut t 21

<210> SEQ ID NO 135
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - KAP.4

<400> SEQUENCE: 135

uacacugcua uggaggacu 19

<210> SEQ ID NO 136
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - KAP.4

<400> SEQUENCE: 136

aguccuccau agcagugua 19

<210> SEQ ID NO 137
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - KAP.4
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 137

uacacugcua uggaggacun n 21

<210> SEQ ID NO 138

-continued

```
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - KAP.4
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 138

nnaguccucc auagcagugu a                                              21


<210> SEQ ID NO 139
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - Prl3.1

<400> SEQUENCE: 139

gugaccuaug acaaaacgct t                                              21


<210> SEQ ID NO 140
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - Prl3.1

<400> SEQUENCE: 140

gugaccuaug acaaaacgc                                                 19


<210> SEQ ID NO 141
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - Prl3.1

<400> SEQUENCE: 141

gcguuuuguc auaggucac                                                 19


<210> SEQ ID NO 142
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - Prl3.1
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 142

gugaccuaug acaaaacgcn n                                              21


<210> SEQ ID NO 143
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - Prl3.1
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
```

-continued

<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 143

nngcguuuug ucauagguca c 21

<210> SEQ ID NO 144
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - Prl3.2

<400> SEQUENCE: 144

ggccaaguuc ugugaggcct t 21

<210> SEQ ID NO 145
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - Prl3.2

<400> SEQUENCE: 145

ggccaaguuc ugugaggcc 19

<210> SEQ ID NO 146
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - Prl3.2

<400> SEQUENCE: 146

ggccucacag aacuuggcc 19

<210> SEQ ID NO 147
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - Prl3.2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 147

ggccaaguuc ugugaggccn n 21

<210> SEQ ID NO 148
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - Prl3.2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 148

nnggccucac agaacuuggc c 21

<210> SEQ ID NO 149

-continued

<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - Prl3.3

<400> SEQUENCE: 149

guacgaggac gccauccagt t 21

<210> SEQ ID NO 150
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - Prl3.3

<400> SEQUENCE: 150

guacgaggac gccauccag 19

<210> SEQ ID NO 151
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - Prl3.3

<400> SEQUENCE: 151

cuggauggcg uccucguac 19

<210> SEQ ID NO 152
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - Prl3.3
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 152

guacgaggac gccauccagn n 21

<210> SEQ ID NO 153
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - Prl3.3
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 153

nncuggaugg cguccucgua c 21

<210> SEQ ID NO 154
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - Prl3.4

<400> SEQUENCE: 154

-continued

```
uaccggccca aacagaggct t                                              21


<210> SEQ ID NO 155
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - Prl3.4

<400> SEQUENCE: 155

uaccggccca aacagaggc                                                 19


<210> SEQ ID NO 156
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - Prl3.4

<400> SEQUENCE: 156

gccucuguuu gggccggua                                                 19


<210> SEQ ID NO 157
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - Prl3.4
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 157

uaccggccca aacagaggcn n                                              21


<210> SEQ ID NO 158
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - Prl3.4
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 158

nngccucugu uugggccggu a                                              21


<210> SEQ ID NO 159
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - RPTPE.1

<400> SEQUENCE: 159

gcagaggaaa gcugugguct t                                              21


<210> SEQ ID NO 160
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
```

-continued

<223> OTHER INFORMATION: Small interfering RNA - RPTPE.1

<400> SEQUENCE: 160

gcagaggaaa gcugugguc 19

<210> SEQ ID NO 161
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - RPTPE.1

<400> SEQUENCE: 161

gaccacagcu uuccucugc 19

<210> SEQ ID NO 162
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - RPTPE.1
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 162

gcagaggaaa gcuguggucn n 21

<210> SEQ ID NO 163
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - RPTPE.1
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 163

nngaccacag cuuuccucug c 21

<210> SEQ ID NO 164
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - RPTPE.2

<400> SEQUENCE: 164

gucugcgacc aucgucaugt t 21

<210> SEQ ID NO 165
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - RPTPE.2

<400> SEQUENCE: 165

gucugcgacc aucgucaug 19

<210> SEQ ID NO 166

-continued

<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - RPTPE.2

<400> SEQUENCE: 166

caugacgaug gucgcagac 19

<210> SEQ ID NO 167
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - RPTPE.2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 167

gucugcgacc aucgucaugn n 21

<210> SEQ ID NO 168
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - RPTPE.2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 168

nncaugacga uggucgcaga c 21

<210> SEQ ID NO 169
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - RPTPE.3

<400> SEQUENCE: 169

gccuuacucg aguacuacct t 21

<210> SEQ ID NO 170
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - RPTPE.3

<400> SEQUENCE: 170

gccuuacucg aguacuacc 19

<210> SEQ ID NO 171
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - RPTPE.3

<400> SEQUENCE: 171

-continued

```
gguaguacuc gaguaaggc                                                  19


<210> SEQ ID NO 172
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - RPTPE.3
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 172

gccuuacucg aguacuaccn n                                               21


<210> SEQ ID NO 173
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - RPTPE.3
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 173

nngguaguac ucgaguaagg c                                               21


<210> SEQ ID NO 174
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - RPTPE.4

<400> SEQUENCE: 174

ggacuauuuc aucgccacct t                                               21


<210> SEQ ID NO 175
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - RPTPE.4

<400> SEQUENCE: 175

ggacuauuuc aucgccacc                                                  19


<210> SEQ ID NO 176
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - RPTPE.4

<400> SEQUENCE: 176

gguggcgaug aaauagucc                                                  19


<210> SEQ ID NO 177
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
```

-continued

```
<223> OTHER INFORMATION: Small interfering RNA - RPTPE.4
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 177

ggacuauuuc aucgccaccn n                                              21


<210> SEQ ID NO 178
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - RPTPE.4
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 178

nngguggcga ugaaauaguc c                                              21


<210> SEQ ID NO 179
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 179

ccaccaucac agcgaacac                                                 19


<210> SEQ ID NO 180
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 180

agcgcuguca uuucaacca                                                 19


<210> SEQ ID NO 181
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 181

accacaacaa uagcuacua                                                 19


<210> SEQ ID NO 182
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 182

gcuacuacuc caucuaagc                                                 19


<210> SEQ ID NO 183
```

-continued

<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 183

aaugcgucug uuuccauau 19

<210> SEQ ID NO 184
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 184

augcgucugu uuccauauc 19

<210> SEQ ID NO 185
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 185

ugcgucuguu uccauaucu 19

<210> SEQ ID NO 186
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 186

accuuuacuu gugauacac 19

<210> SEQ ID NO 187
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 187

cagauuucag ugugguaau 19

<210> SEQ ID NO 188
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 188

acccgaacau gaguauaag 19

<210> SEQ ID NO 189
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

-continued

<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 189

cccgaacaug aguauaagu 19

<210> SEQ ID NO 190
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 190

caaguuuacu aacgcaagu 19

<210> SEQ ID NO 191
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 191

ggaguaauua ccuggaauc 19

<210> SEQ ID NO 192
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 192

caugccuaca ucauugcaa 19

<210> SEQ ID NO 193
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 193

auaguaugca ugucaagug 19

<210> SEQ ID NO 194
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 194

ugaacguuac cauuuggaa 19

<210> SEQ ID NO 195
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 195

-continued

```
augagucgca uaagaauug                                               19


<210> SEQ ID NO 196
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 196

ugagucgcau aagaauugc                                               19


<210> SEQ ID NO 197
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 197

gaauugcgau uuccgugua                                               19


<210> SEQ ID NO 198
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 198

auugcgauuu ccguguaaa                                               19


<210> SEQ ID NO 199
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 199

gccaauccau gcagauauu                                               19


<210> SEQ ID NO 200
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 200

uuauaaccgu guugaacuc                                               19


<210> SEQ ID NO 201
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 201

uaaccguguu gaacucucu                                               19


<210> SEQ ID NO 202
```

-continued

```
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 202

acggagaugc agggucaaa                                                   19


<210> SEQ ID NO 203
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 203

gaugcagggu caaacuaca                                                   19


<210> SEQ ID NO 204
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 204

acccaggaaa uacauugcu                                                   19


<210> SEQ ID NO 205
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 205

uguccagauu acaucauuc                                                   19


<210> SEQ ID NO 206
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 206

augccuucag caauuucuu                                                   19


<210> SEQ ID NO 207
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 207

caggaaccua uaucggaau                                                   19


<210> SEQ ID NO 208
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
```

-continued

<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 208

ggaaccuaua ucggaauug 19

<210> SEQ ID NO 209
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 209

accuauaucg gaauugaug 19

<210> SEQ ID NO 210
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 210

guggauguuu augguuaug 19

<210> SEQ ID NO 211
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 211

ggcgacagag augccugau 19

<210> SEQ ID NO 212
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 212

gaggcccagu acaucuuga 19

<210> SEQ ID NO 213
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 213

ggcccaguac aucuugauc 19

<210> SEQ ID NO 214
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 214

-continued

gcuacuggaa accugaagu 19

<210> SEQ ID NO 215
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 215

accugaagug augauugcu 19

<210> SEQ ID NO 216
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 216

aguugaccug aaagacaca 19

<210> SEQ ID NO 217
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 217

acuuauaccc uucgugucu 19

<210> SEQ ID NO 218
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 218

cuuauacccu ucgugucuu 19

<210> SEQ ID NO 219
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 219

ggaaagacuc ucgaacugu 19

<210> SEQ ID NO 220
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 220

acccaaggaa uuaaucucu 19

<210> SEQ ID NO 221

-continued

<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 221

cccaaggaau uaaucucua 19

<210> SEQ ID NO 222
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 222

ugauucaggu cgucaaaca 19

<210> SEQ ID NO 223
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 223

gggauggauc ucagcaaac 19

<210> SEQ ID NO 224
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 224

ucucagcaaa cgggaauau 19

<210> SEQ ID NO 225
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 225

uucgagcaau aucaauucc 19

<210> SEQ ID NO 226
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 226

ccuacccugc ucagaaugg 19

<210> SEQ ID NO 227
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

-continued

<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 227

auggagcugu cacccacau 19

<210> SEQ ID NO 228
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 228

uggaacauca cgggcaauu 19

<210> SEQ ID NO 229
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 229

gcaaugacgg caagucuaa 19

SEQ ID NO 2 30
<211> LENGTH: 19
<212> TYPE: RNA
ORGANISM: Artificial Sequen ce
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 230

augacggcaa gucuaaagu 19

<210> SEQ ID NO 231
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 231

ugacggcaag ucuaaagug 19

<210> SEQ ID NO 232
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 232

gucuaaagug acccauguu 19

<210> SEQ ID NO 233
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 233

-continued

ugauucgcug ucaggaacu 19

<210> SEQ ID NO 234
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 234

cgacguuggu ggaggagaa 19

<210> SEQ ID NO 235
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 235

acgguuugau ucuuugaca 19

<210> SEQ ID NO 236
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 236

uucuuugaca gaucuugug 19

<210> SEQ ID NO 237
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 237

gaauccuaug guggaaaca 19

<210> SEQ ID NO 238
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 238

auccuauggu ggaaacauu 19

<210> SEQ ID NO 239
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 239

uccuauggug gaaacauug 19

<210> SEQ ID NO 240

-continued

<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 240

caguacuaca acucaagca 19

<210> SEQ ID NO 241
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 241

uuugagacac uacaacaac 19

<210> SEQ ID NO 242
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 242

aacuucucua cagccgaaa 19

<210> SEQ ID NO 243
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 243

acauccugcc cuuugauca 19

<210> SEQ ID NO 244
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 244

ucauaccagg guuguccua 19

<210> SEQ ID NO 245
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 245

uaccaggguu guccuacac 19

<210> SEQ ID NO 246
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

-continued

<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 246

uuugaaacca agugcaaca 19

<210> SEQ ID NO 247
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 247

agaguuacau ugccacaca 19

<210> SEQ ID NO 248
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 248

gaguuacauu gccacacaa 19

<210> SEQ ID NO 249
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 249

aaacacggug aaugacuuu 19

<210> SEQ ID NO 250
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 250

cuggccugau gaguaugcu 19

<210> SEQ ID NO 251
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 251

uggcgucaug cguguuagg 19

<210> SEQ ID NO 252
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 252

-continued

ugcguguuag gaacgucaa 19

<210> SEQ ID NO 253
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 253

ugacuauacg cuaagagaa 19

<210> SEQ ID NO 254
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 254

cuauacgcua agagaacuu 19

<210> SEQ ID NO 255
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 255

gguuggacaa gggaauacg 19

<210> SEQ ID NO 256
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 256

gaacggucug gcaauacca 19

<210> SEQ ID NO 257
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 257

cggucuggca auaccacuu 19

<210> SEQ ID NO 258
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 258

aagguguuga cugcgauau 19

<210> SEQ ID NO 259

-continued

<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 259

agguguugac ugcgauauu 19

<210> SEQ ID NO 260
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 260

gguguugacu gcgauauug 19

<210> SEQ ID NO 261
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 261

uauggcgguc cagcauuau 19

<210> SEQ ID NO 262
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 262

uggcggucca gcauuauau 19

<210> SEQ ID NO 263
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 263

aacacuacag cgcaggauu 19

<210> SEQ ID NO 264
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 264

acacuacagc gcaggauug 19

<210> SEQ ID NO 265
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

-continued

<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 265

gcgcaggauu gaagaagag 19

<210> SEQ ID NO 266
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 266

gaggaaaggg cacgaauau 19

<210> SEQ ID NO 267
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 267

ggaaagggca cgaauauac 19

<210> SEQ ID NO 268
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 268

gggcacgaau auacaaaua 19

<210> SEQ ID NO 269
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 269

aaacgugggc cugaugcaa 19

<210> SEQ ID NO 270
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 270

acgugggccu gaugcaaca 19

<210> SEQ ID NO 271
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 271

-continued

```
gcacaguaaa uacccacua                                                   19


<210> SEQ ID NO 272
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 272

cuauuucucc aucgaugag                                                   19


<210> SEQ ID NO 273
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 273

acuuggcaau gguguacag                                                   19


<210> SEQ ID NO 274
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 274

ggugccuaug caguaaucu                                                   19


<210> SEQ ID NO 275
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 275

ucucaccauu cucgacugu                                                   19


<210> SEQ ID NO 276
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 276

aagggauuac aacauggau                                                   19


<210> SEQ ID NO 277
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 277

agggauuaca acauggauu                                                   19


<210> SEQ ID NO 278
```

-continued

<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 278

gggauuacaa cauggauuu 19

<210> SEQ ID NO 279
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 279

gaaugguuau ccucuucac 19

<210> SEQ ID NO 280
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 280

gcauaaugug acugcaguu 19

<210> SEQ ID NO 281
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 281

cgcuggcuuc gagcacuau 19

<210> SEQ ID NO 282
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 282

gcacacccag ugacaacau 19

<210> SEQ ID NO 283
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 283

acaucgugcg aagguuccu 19

<210> SEQ ID NO 284
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

-continued

<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 284

agaacaggga cauugauag 19

<210> SEQ ID NO 285
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 285

gaacagggac auugauagc 19

<210> SEQ ID NO 286
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 286

gggacauuga uagccuguu 19

<210> SEQ ID NO 287
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 287

cauugauagc cuguuaugu 19

<210> SEQ ID NO 288
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 288

cuacagguuu acacaugcu 19

<210> SEQ ID NO 289
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 289

aaaucgacca uccagugaa 19

<210> SEQ ID NO 290
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 290

-continued

aaucgaccau ccagugaag 19

<210> SEQ ID NO 291
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 291

ucgaccaucc agugaagga 19

<210> SEQ ID NO 292
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 292

aaauucuuuc uggccuaga 19

<210> SEQ ID NO 293
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 293

ugucuauugg uggaaaucu 19

<210> SEQ ID NO 294
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 294

acgauuugga gagguaagu 19

<210> SEQ ID NO 295
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 295

cgauuuggag agguaaguu 19

<210> SEQ ID NO 296
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 296

gagacauccu auauuccuu 19

<210> SEQ ID NO 297

-continued

<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 297

auaccagacc gauuuauug 19

<210> SEQ ID NO 298
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 298

uaccagaccg auuuauugc 19

<210> SEQ ID NO 299
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 299

gaccgauuua uugccuucu 19

<210> SEQ ID NO 300
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 300

aaggauguau gaugccaaa 19

<210> SEQ ID NO 301
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 301

aggauguaug augccaaac 19

<210> SEQ ID NO 302
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 302

ggauguauga ugccaaacg 19

<210> SEQ ID NO 303
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

-continued

<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 303

cggaugcugg cuucgauca 19

<210> SEQ ID NO 304
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 304

ugccauuguc aaagaauuc 19

<210> SEQ ID NO 305
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 305

gggugccauu gcaguacau 19

<210> SEQ ID NO 306
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 306

gaccuggcuc ggugauugg 19

<210> SEQ ID NO 307
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 307

cccgaaccgu acagugaug 19

<210> SEQ ID NO 308
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 308

accguacagu gaugaugac 19

<210> SEQ ID NO 309
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 309

-continued

```
uagacuucgg gccuugaaa                                                    19


<210> SEQ ID NO 310
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 310

acaaacgcua uuccucuca                                                    19


<210> SEQ ID NO 311
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 311

gggucugggc agugauuau                                                    19


<210> SEQ ID NO 312
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 312

gcaaccacug gaggugaag                                                    19


<210> SEQ ID NO 313
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 313

auccuaugag aagaauaca                                                    19


<210> SEQ ID NO 314
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 314

uccuaugaga agaauacau                                                    19


<210> SEQ ID NO 315
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 315

aaagcuguug ggauguagu                                                    19


<210> SEQ ID NO 316
```

-continued

<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 316

uucugauucu cuugaccau 19

<210> SEQ ID NO 317
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 317

gaagccagua agaccugua 19

<210> SEQ ID NO 318
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 318

cagccacuuu gucugauga 19

<210> SEQ ID NO 319
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 319

aaccuugaca accgaugca 19

<210> SEQ ID NO 320
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 320

caaccgaugc aagcuguuu 19

<210> SEQ ID NO 321
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 321

accgaugcaa gcuguuuga 19

<210> SEQ ID NO 322
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

-continued

<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 322

cucggucagu guugaagag 19

<210> SEQ ID NO 323
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 323

acguucucaa gaggagucu 19

<210> SEQ ID NO 324
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 324

gucaacuaau ccagagaag 19

<210> SEQ ID NO 325
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 325

aggcccauga gacucuuca 19

<210> SEQ ID NO 326
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 326

agggaccuua uaggagacu 19

<210> SEQ ID NO 327
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 327

gggaccuuau aggagacuu 19

<210> SEQ ID NO 328
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 328

-continued

gacuucucca aggguuauc 19

<210> SEQ ID NO 329
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 329

guuuguuauc aucgacugu 19

<210> SEQ ID NO 330
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 330

cugucgauac ccauaugaa 19

<210> SEQ ID NO 331
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 331

gaagcccauu guaccuacu 19

<210> SEQ ID NO 332
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 332

agcccauugu accuacuga 19

<210> SEQ ID NO 333
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 333

gcccauugua ccuacugau 19

<210> SEQ ID NO 334
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 334

uggcaagcgu gucauuguu 19

<210> SEQ ID NO 335

-continued

<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 335

agcgugucau uguuguguu 19

<210> SEQ ID NO 336
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 336

ugugccggua ugugagaga 19

<210> SEQ ID NO 337
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 337

gagagaucgc cuggguaau 19

<210> SEQ ID NO 338
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 338

gagaucgccu ggguaauga 19

<210> SEQ ID NO 339
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 339

gaucgccugg guaaugaau 19

<210> SEQ ID NO 340
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 340

auccucccug ucgucugaa 19

<210> SEQ ID NO 341
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

-continued

<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 341

uccucccugu cgucugaau 19

<210> SEQ ID NO 342
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 342

uggcggagca gacguuuga 19

<210> SEQ ID NO 343
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 343

cguuugaaca ggccaucca 19

<210> SEQ ID NO 344
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 344

gccggaucau ucgaaacga 19

<210> SEQ ID NO 345
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 345

ucauucgaaa cgagcaguu 19

<210> SEQ ID NO 346
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 346

gucuaugccg gauggauuu 19

<210> SEQ ID NO 347
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 347

-continued

ugccggaugg auuugucuu 19

<210> SEQ ID NO 348
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 348

aaaggaccuc gucauguac 19

<210> SEQ ID NO 349
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 349

aaucacugug ucacgauga 19

<210> SEQ ID NO 350
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 350

aucacugugu cacgaugag 19

<210> SEQ ID NO 351
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 351

gagcugauug gagauuacu 19

<210> SEQ ID NO 352
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 352

gcugauugga gauuacucu 19

<210> SEQ ID NO 353
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 353

cucuaaggcc uuccuccua 19

<210> SEQ ID NO 354

-continued

<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 354

cagacaguag acggaaagc 19

<210> SEQ ID NO 355
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 355

agcaccaaga ccucaagua 19

<210> SEQ ID NO 356
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 356

gaaacgaugg uggcccuau 19

<210> SEQ ID NO 357
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 357

aacgauggug gcccuauug 19

<210> SEQ ID NO 358
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 358

cgccgagagc uuccuacug 19

<210> SEQ ID NO 359
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 359

gaacuccagu gggcaaauu 19

<210> SEQ ID NO 360
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

-continued

<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 360

uuuagcuggg augacaaug 19

<210> SEQ ID NO 361
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 361

uucaaggaca acacaauac 19

<210> SEQ ID NO 362
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 362

acacaauacc agauaaagu 19

<210> SEQ ID NO 363
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 363

cacaauacca gauaaaguu 19

<210> SEQ ID NO 364
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 364

ggaagggcuu auguuuaaa 19

<210> SEQ ID NO 365
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 365

caccaagauc ugaaguaug 19

<210> SEQ ID NO 366
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 366

-continued

aguaugucaa cccagaaac 19

<210> SEQ ID NO 367
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 367

guaugucaac ccagaaaca 19

<210> SEQ ID NO 368
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 368

ugucauugau ugucgcuau 19

<210> SEQ ID NO 369
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 369

uugauugucg cuauccaua 19

<210> SEQ ID NO 370
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 370

uugucgcuau ccauaugag 19

<210> SEQ ID NO 371
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 371

uccagggagc cuuaaacuu 19

<210> SEQ ID NO 372
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 372

gggagccuua aacuuauau 19

<210> SEQ ID NO 373

-continued

<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 373

gucaggaaga acuguuuaa 19

<210> SEQ ID NO 374
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 374

agaagcccau cgucccuuu 19

<210> SEQ ID NO 375
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 375

gaagcccauc gucccuuug 19

<210> SEQ ID NO 376
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 376

agcccaucgu cccuuugga 19

<210> SEQ ID NO 377
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 377

cacccagaag agaauaauc 19

<210> SEQ ID NO 378
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 378

uuguacuacc cagagcuau 19

<210> SEQ ID NO 379
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

-continued

<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 379

cuacccagag cuauauauc 19

<210> SEQ ID NO 380
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 380

cccagagcua uauauccuu 19

<210> SEQ ID NO 381
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 381

uauauggaac ugugugaac 19

<210> SEQ ID NO 382
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 382

uauggaacug ugugaacca 19

<210> SEQ ID NO 383
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 383

cagagcuacu gcccuaugc 19

<210> SEQ ID NO 384
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 384

gagcuacugc ccuaugcau 19

<210> SEQ ID NO 385
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 385

-continued

gcuacugccc uaugcauca 19

<210> SEQ ID NO 386
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 386

gaugaagagc cuauugaag 19

<210> SEQ ID NO 387
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 387

agaugaacag acuccaauu 19

<210> SEQ ID NO 388
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 388

gaugaacaga cuccaauuc 19

<210> SEQ ID NO 389
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 389

ucacccauca ucauccaau 19

<210> SEQ ID NO 390
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 390

gagcuuacaa ccugccuua 19

<210> SEQ ID NO 391
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 391

cacugcuaug gaggacuug 19

<210> SEQ ID NO 392

-continued

<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 392

ucaccagagc aagccauag 19

<210> SEQ ID NO 393
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 393

ccagagcaag ccauagaca 19

<210> SEQ ID NO 394
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 394

cagccugcga gaccuaaga 19

<210> SEQ ID NO 395
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 395

guuucgggac aaauuagcu 19

<210> SEQ ID NO 396
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 396

aauuagcugc acaucuauc 19

<210> SEQ ID NO 397
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 397

auuagcugca caucuauca 19

<210> SEQ ID NO 398
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

-continued

<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 398

uuagcugcac aucuaucau 19

<210> SEQ ID NO 399
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 399

gagacgcgga acaauugag 19

<210> SEQ ID NO 400
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 400

agaacaaggu gacacauau 19

<210> SEQ ID NO 401
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 401

gaacaaggug acacauauu 19

<210> SEQ ID NO 402
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 402

gcagcggauu caccaucuc 19

<210> SEQ ID NO 403
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 403

gcggauucac caucucaaa 19

<210> SEQ ID NO 404
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 404

-continued

cacuggugau cgcauacau 19

<210> SEQ ID NO 405
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 405

guaucggcag uggcugaag 19

<210> SEQ ID NO 406
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 406

gauccgccga cgacugcaa 19

<210> SEQ ID NO 407
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 407

guuucgggag gaguucaac 19

<210> SEQ ID NO 408
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 408

augaccauuc uagggugau 19

<210> SEQ ID NO 409
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 409

ccauucuagg gugauucug 19

<210> SEQ ID NO 410
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 410

cauagauggu uacaaagag 19

<210> SEQ ID NO 411

-continued

<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 411

aacaggaaac gguuaacga 19

<210> SEQ ID NO 412
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 412

ggaaacgguu aacgacuuc 19

<210> SEQ ID NO 413
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 413

ccaucgucau guuaacaaa 19

<210> SEQ ID NO 414
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 414

cuacaccauc cggaaguuc 19

<210> SEQ ID NO 415
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 415

uccggaaguu cugcauaca 19

<210> SEQ ID NO 416
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 416

gaaaguaaag acgcucaac 19

<210> SEQ ID NO 417
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

-continued

<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 417

gcgcccucag augguucaa 19

<210> SEQ ID NO 418
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 418

cggauaugca guacacguu 19

<210> SEQ ID NO 419
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 419

ccacccacuu cgacaagau 19

<210> SEQ ID NO 420
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 420

caaauguccg gaucaugaa 19

<210> SEQ ID NO 421
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 421

caugaggacg ggcaacuug 19

<210> SEQ ID NO 422
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 422

ugacuucaac cgagugauc 19

<210> SEQ ID NO 423
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 423

-continued

accgagugau ccuuuccau 19

<210> SEQ ID NO 424
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 424

agaauacaca gacuacauc 19

<210> SEQ ID NO 425
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 425

gacuacauca acgcauccu 19

<210> SEQ ID NO 426
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 426

ucaacgcauc cuucauaga 19

<210> SEQ ID NO 427
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 427

cacacgguug aggacuucu 19

<210> SEQ ID NO 428
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 428

aaucccacac uaucgugau 19

<210> SEQ ID NO 429
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 429

aucccacacu aucgugaug 19

<210> SEQ ID NO 430

-continued

<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 430

accgagggcu caguuacuc 19

<210> SEQ ID NO 431
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 431

ccgagggcuc aguuacuca 19

<210> SEQ ID NO 432
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 432

cucauggaga aauaacgau 19

<210> SEQ ID NO 433
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 433

uggagaaaua acgauugag 19

<210> SEQ ID NO 434
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 434

gccaucagua uacgagacu 19

<210> SEQ ID NO 435
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 435

ucaguauacg agacuuucu 19

<210> SEQ ID NO 436
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

-continued

<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 436

gggcaaaggc augauugac 19

<210> SEQ ID NO 437
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 437

gcugggcgaa cagguacau 19

<210> SEQ ID NO 438
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA

<400> SEQUENCE: 438

cuucagagac cacauaugg 19

<210> SEQ ID NO 439
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc14a.2

<400> SEQUENCE: 439

caucugugag aacaccgaat t 21

<210> SEQ ID NO 440
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc14a.2

<400> SEQUENCE: 440

caucugugag aacaccgaa 19

<210> SEQ ID NO 441
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc14a.2

<400> SEQUENCE: 441

uucgguguuc ucacagaug 19

<210> SEQ ID NO 442
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc14a.2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21

-continued

<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 442

caucugugag aacaccgaan n 21

<210> SEQ ID NO 443
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc14a.2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 443

nnuucggugu ucucacagau g 21

<210> SEQ ID NO 444
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc14a.3

<400> SEQUENCE: 444

cuuggcaaug guguacagat t 21

<210> SEQ ID NO 445
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc14a.3

<400> SEQUENCE: 445

cuuggcaaug guguacaga 19

<210> SEQ ID NO 446
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc14a.3

<400> SEQUENCE: 446

ucuguacacc auugccaag 19

<210> SEQ ID NO 447
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc14a.3
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 447

cuuggcaaug guguacagan n 21

<210> SEQ ID NO 448

-continued

<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc14a.3
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 448

nnucuguaca ccauugccaa g 21

<210> SEQ ID NO 449
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc14a.5

<400> SEQUENCE: 449

gcacaguaaa uacccacuat t 21

<210> SEQ ID NO 450
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc14a.5

<400> SEQUENCE: 450

gcacaguaaa uacccacua 19

<210> SEQ ID NO 451
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc14a.5

<400> SEQUENCE: 451

uaguggguau uuacugugc 19

<210> SEQ ID NO 452
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc14a.5
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 452

gcacaguaaa uacccacuan n 21

<210> SEQ ID NO 453
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc14a.5
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2

-continued

<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 453

nnuagugggu auuuacugug c 21

<210> SEQ ID NO 454
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc14b.3

<400> SEQUENCE: 454

caagcaaaug cugccuucct t 21

<210> SEQ ID NO 455
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc14b.3

<400> SEQUENCE: 455

caagcaaaug cugccuucc 19

<210> SEQ ID NO 456
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc14b.3

<400> SEQUENCE: 456

ggaaggcagc auuugcuug 19

<210> SEQ ID NO 457
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc14b.3
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 457

caagcaaaug cugccuuccn n 21

<210> SEQ ID NO 458
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc14b.3
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 458

nnggaaggca gcauuugcuu g 21

<210> SEQ ID NO 459

-continued

<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc14b.4

<400> SEQUENCE: 459

gagccagacu ugaaaguggt t 21

<210> SEQ ID NO 460
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc14b.4

<400> SEQUENCE: 460

gagccagacu ugaaagugg 19

<210> SEQ ID NO 461
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc14b.4

<400> SEQUENCE: 461

ccacuuucaa gucuggcuc 19

<210> SEQ ID NO 462
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc14b.4
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 462

gagccagacu ugaaaguggn n 21

<210> SEQ ID NO 463
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc14b.4
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 463

nnccacuuuc aagucuggcu c 21

<210> SEQ ID NO 464
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - 25 A.2

<400> SEQUENCE: 464

-continued

gaggagccau ucugauucut t 21

<210> SEQ ID NO 465
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - 25 A.2

<400> SEQUENCE: 465

gaggagccau ucugauucu 19

<210> SEQ ID NO 466
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - 25 A.2

<400> SEQUENCE: 466

agaaucagaa uggcuccuc 19

<210> SEQ ID NO 467
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - 25 A.2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 467

gaggagccau ucugauucun n 21

<210> SEQ ID NO 468
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - 25 A.2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 468

nnagaaucag aauggcuccu c 21

<210> SEQ ID NO 469
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc25B.2

<400> SEQUENCE: 469

aggcggcuac aaggaguuct t 21

<210> SEQ ID NO 470
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

-continued

<223> OTHER INFORMATION: Small interfering RNA - cdc25B.2

<400> SEQUENCE: 470

aggcggcuac aaggaguuc 19

<210> SEQ ID NO 471
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc25B.2

<400> SEQUENCE: 471

gaacuccuug uagccgccu 19

<210> SEQ ID NO 472
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc25B.2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 472

aggcggcuac aaggaguucn n 21

<210> SEQ ID NO 473
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc25B.2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 473

nngaacuccu uguagccgcc u 21

<210> SEQ ID NO 474
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc25B.4

<400> SEQUENCE: 474

gaugccaugg aagcccacat t 21

<210> SEQ ID NO 475
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc25B.4

<400> SEQUENCE: 475

gaugccaugg aagcccaca 19

<210> SEQ ID NO 476

-continued

<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc25B.4

<400> SEQUENCE: 476

ugugggcuuc cauggcauc 19

<210> SEQ ID NO 477
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc25B.4
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 477

gaugccaugg aagcccacan n 21

<210> SEQ ID NO 478
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc25B.4
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 478

nnugugggcu uccauggcau c 21

<210> SEQ ID NO 479
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc25C.1

<400> SEQUENCE: 479

cugccacuca gcuuaccact t 21

<210> SEQ ID NO 480
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc25C.1

<400> SEQUENCE: 480

cugccacuca gcuuaccac 19

<210> SEQ ID NO 481
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc25C.1

<400> SEQUENCE: 481

-continued

gugguaagcu gaguggcag 19

<210> SEQ ID NO 482
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc25C.1
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 482

cugccacuca gcuuaccacn n 21

<210> SEQ ID NO 483
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc25C.1
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 483

nnugguaag cugaguggca g 21

<210> SEQ ID NO 484
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc25C.3

<400> SEQUENCE: 484

cccagaaaca guggcugcct t 21

<210> SEQ ID NO 485
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc25C.3

<400> SEQUENCE: 485

cccagaaaca guggcugcc 19

<210> SEQ ID NO 486
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc25C.3

<400> SEQUENCE: 486

ggcagccacu guuucuggg 19

<210> SEQ ID NO 487
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

-continued

<223> OTHER INFORMATION: Small interfering RNA - cdc25C.3
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 487

cccagaaaca guggcugccn n 21

<210> SEQ ID NO 488
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc25C.3
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 488

nnggcagcca cuguuucugg g 21

<210> SEQ ID NO 489
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc25C.4

<400> SEQUENCE: 489

aggcggcuac agagacuuct t 21

<210> SEQ ID NO 490
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc25C.4

<400> SEQUENCE: 490

aggcggcuac agagacuuc 19

<210> SEQ ID NO 491
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc25C.4

<400> SEQUENCE: 491

gaagucucug uagccgccu 19

<210> SEQ ID NO 492
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc25C.4
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 492

-continued

aggcggcuac agagacuucn n 21

<210> SEQ ID NO 493
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - cdc25C.4
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 493

nngaagucuc uguagccgcc u 21

<210> SEQ ID NO 494
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer mPTP1B-sense

<400> SEQUENCE: 494

ggggggatc catggagatg gagaaggagt tcgagg 36

<210> SEQ ID NO 495
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer mPTP1B anti-sense

<400> SEQUENCE: 495

gggggaattc tcagtgaaaa cacacccggt agcac 35

<210> SEQ ID NO 496
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - mPTP1B1.1

<400> SEQUENCE: 496

gaagcccaga ggagcuauat t 21

<210> SEQ ID NO 497
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - mPTP1B1.1

<400> SEQUENCE: 497

gaagcccaga ggagcuaua 19

<210> SEQ ID NO 498
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - mPTP1B1.1

<400> SEQUENCE: 498

-continued

uauagcuccu cugggcuuc 19

<210> SEQ ID NO 499
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - mPTP1B1.1
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 499

gaagcccaga ggagcuauan n 21

<210> SEQ ID NO 500
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - mPTP1B1.1
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 500

nnuauagcuc cucugggcuu c 21

<210> SEQ ID NO 501
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - mPTP1B1.2

<400> SEQUENCE: 501

cuacaccaca uggccugact t 21

<210> SEQ ID NO 502
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - mPTP1B1.2

<400> SEQUENCE: 502

cuacaccaca uggccugac 19

<210> SEQ ID NO 503
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - mPTP1B1.2

<400> SEQUENCE: 503

gucaggccau gugguguag 19

<210> SEQ ID NO 504
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

-continued

<223> OTHER INFORMATION: Small interfering RNA - mPTP1B1.2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 504

cuacaccaca uggccugacn n 21

<210> SEQ ID NO 505
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - mPTP1B1.2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 505

nngucaggcc auguggugua g 21

<210> SEQ ID NO 506
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - mPTP1B1.3

<400> SEQUENCE: 506

gacugccgac cagcugcgct t 21

<210> SEQ ID NO 507
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - mPTP1B1.3

<400> SEQUENCE: 507

gacugccgac cagcugcgc 19

<210> SEQ ID NO 508
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - mPTP1B1.3

<400> SEQUENCE: 508

gcgcagcugg ucggcaguc 19

<210> SEQ ID NO 509
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - mPTP1B1.3
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 509

-continued

```
gacugccgac cagcugcgcn n                                                21


<210> SEQ ID NO 510
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - mPTP1B1.3
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 510

nngcgcagcu ggucggcagu c                                                21


<210> SEQ ID NO 511
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - mPTP1B1.4

<400> SEQUENCE: 511

gguaccgaga ugucagccct t                                                21


<210> SEQ ID NO 512
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - mPTP1B1.4

<400> SEQUENCE: 512

gguaccgaga ugucagccc                                                   19


<210> SEQ ID NO 513
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - mPTP1B1.4

<400> SEQUENCE: 513

gggcugacau cucgguacc                                                   19


<210> SEQ ID NO 514
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - mPTP1B1.4
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 514

gguaccgaga ugucagcccn n                                                21


<210> SEQ ID NO 515
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
```

-continued

<223> OTHER INFORMATION: Small interfering RNA - mPTP1B1.4
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 515

nngggcugac aucucgguac c 21

<210> SEQ ID NO 516
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - mPTP1B1.5

<400> SEQUENCE: 516

ugacuauauc aaugccagct t 21

<210> SEQ ID NO 517
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - mPTP1B1.5

<400> SEQUENCE: 517

ugacuauauc aaugccagc 19

<210> SEQ ID NO 518
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - mPTP1B1.5

<400> SEQUENCE: 518

gcuggcauug auauaguca 19

<210> SEQ ID NO 519
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - mPTP1B1.5
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 519

ugacuauauc aaugccagcn n 21

<210> SEQ ID NO 520
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - mPTP1B1.5
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 520

-continued

```
nngcuggcau ugauauaguc a                                               21


<210> SEQ ID NO 521
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - mPTP1B1.6

<400> SEQUENCE: 521

agaagaaaag gagauggguct t                                              21


<210> SEQ ID NO 522
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - mPTP1B1.6

<400> SEQUENCE: 522

agaagaaaag gagaugguc                                                  19


<210> SEQ ID NO 523
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - mPTP1B1.6

<400> SEQUENCE: 523

gaccaucucc uuuucuucu                                                  19


<210> SEQ ID NO 524
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - mPTP1B1.6
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 524

agaagaaaag gagauggucn n                                               21


<210> SEQ ID NO 525
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - mPTP1B1.6
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 525

nngaccaucu ccuuuucuuc u                                               21


<210> SEQ ID NO 526
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
```

-continued

<223> OTHER INFORMATION: Small interfering RNA - mPTP1B1.7

<400> SEQUENCE: 526

cgggaagugc aaggagcuct t 21

<210> SEQ ID NO 527
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - mPTP1B1.7

<400> SEQUENCE: 527

cgggaagugc aaggagcuc 19

<210> SEQ ID NO 528
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - mPTP1B1.7

<400> SEQUENCE: 528

gagcuccuug cacuucccg 19

<210> SEQ ID NO 529
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - mPTP1B1.7
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 529

cgggaagugc aaggagcucn n 21

<210> SEQ ID NO 530
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - mPTP1B1.7
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 530

nngagcuccu ugcacuuccc g 21

<210> SEQ ID NO 531
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - mPTP1B1.8

<400> SEQUENCE: 531

ggaucagugg aaggagcuct c 21

<210> SEQ ID NO 532

-continued

<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - mPTP1B1.8

<400> SEQUENCE: 532

ggaucagugg aaggagcuc 19

<210> SEQ ID NO 533
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - mPTP1B1.8

<400> SEQUENCE: 533

gagcuccuuc cacugaucc 19

<210> SEQ ID NO 534
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - mPTP1B1.8
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 534

ggaucagugg aaggagcucn n 21

<210> SEQ ID NO 535
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - mPTP1B1.8
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 535

nngagcuccu uccacugauc c 21

<210> SEQ ID NO 536
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - rPTP1B1.1

<400> SEQUENCE: 536

agaagaaaaa gagaugguct t 21

<210> SEQ ID NO 537
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - rPTP1B1.1

<400> SEQUENCE: 537

-continued

```
agaagaaaaa gagaugguc                                                    19


<210> SEQ ID NO 538
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - rPTP1B1.1

<400> SEQUENCE: 538

gaccaucucu uuuucuucu                                                    19


<210> SEQ ID NO 539
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - rPTP1B1.1
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 539

agaagaaaaa gagauggucn n                                                 21


<210> SEQ ID NO 540
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - rPTP1B1.1
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 540

nngaccaucu cuuuuucuuc u                                                 21


<210> SEQ ID NO 541
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - rPTP1B1.2

<400> SEQUENCE: 541

cggauggugg guggagguct t                                                 21


<210> SEQ ID NO 542
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - rPTP1B1.2

<400> SEQUENCE: 542

cggauggugg guggagguc                                                    19


<210> SEQ ID NO 543
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
```

-continued

<223> OTHER INFORMATION: Small interfering RNA - rPTP1B1.2

<400> SEQUENCE: 543

gaccuccacc caccauccg 19

<210> SEQ ID NO 544
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - rPTP1B1.2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 544

cggauggugg guggaggucn n 21

<210> SEQ ID NO 545
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - rPTP1B1.2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 545

nngaccucca cccaccaucc g 21

<210> SEQ ID NO 546
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - rPTP1B1.3

<400> SEQUENCE: 546

uggcaagugc aaggagcuct t 21

<210> SEQ ID NO 547
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - rPTP1B1.3

<400> SEQUENCE: 547

uggcaagugc aaggagcuc 19

<210> SEQ ID NO 548
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - rPTP1B1.3

<400> SEQUENCE: 548

gagcuccuug cacuugcca 19

<210> SEQ ID NO 549

-continued

<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - rPTP1B1.3
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 549

uggcaagugc aaggagcucn n 21

<210> SEQ ID NO 550
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - rPTP1B1.3
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 550

nngagcuccu ugcacuugcc a 21

<210> SEQ ID NO 551
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - rPTP1B1.4

<400> SEQUENCE: 551

cuacaccacc uggccugact t 21

<210> SEQ ID NO 552
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - rPTP1B1.4

<400> SEQUENCE: 552

cuacaccacc uggccugac 19

<210> SEQ ID NO 553
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - rPTP1B1.4

<400> SEQUENCE: 553

gucaggccag gugguguag 19

<210> SEQ ID NO 554
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - rPTP1B1.4
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21

-continued

<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 554

cuacaccacc uggccugacn n 21

<210> SEQ ID NO 555
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - rPTP1B1.4
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 555

nngucaggcc agguggugua g 21

<210> SEQ ID NO 556
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hPTP1B1.1

<400> SEQUENCE: 556

cuauaccaca uggccugact t 21

<210> SEQ ID NO 557
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hPTP1B1.1

<400> SEQUENCE: 557

cuauaccaca uggccugac 19

<210> SEQ ID NO 558
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hPTP1B1.1

<400> SEQUENCE: 558

gucaggccau gugguauag 19

<210> SEQ ID NO 559
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hPTP1B1.1
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 559

cuauaccaca uggccugacn n 21

<210> SEQ ID NO 560

-continued

<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hPTP1B1.1
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 560

nngucaggcc augugguaua g 21

<210> SEQ ID NO 561
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hPTP1B1.2

<400> SEQUENCE: 561

gcccaaagga guuacauuct t 21

<210> SEQ ID NO 562
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hPTP1B1.2

<400> SEQUENCE: 562

gcccaaagga guuacauuc 19

<210> SEQ ID NO 563
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hPTP1B1.2

<400> SEQUENCE: 563

gaauguaacu ccuuugggc 19

<210> SEQ ID NO 564
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hPTP1B1.2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 564

gcccaaagga guuacauucn n 21

<210> SEQ ID NO 565
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hPTP1B1.2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2

-continued

<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 565

nngaauguaa cuccuuuggg c 21

<210> SEQ ID NO 566
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hPTP1B1.3

<400> SEQUENCE: 566

ggaagaaaaa ggaagcccct t 21

<210> SEQ ID NO 567
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hPTP1B1.3

<400> SEQUENCE: 567

ggaagaaaaa ggaagcccc 19

<210> SEQ ID NO 568
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hPTP1B1.3

<400> SEQUENCE: 568

ggggcuuccu uuuucuucc 19

<210> SEQ ID NO 569
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hPTP1B1.3
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 569

ggaagaaaaa ggaagccccn n 21

<210> SEQ ID NO 570
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hPTP1B1.3
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 570

nnggggcuuc cuuuuucuuc c 21

<210> SEQ ID NO 571

-continued

<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hPTP1B1.4

<400> SEQUENCE: 571

caaugggaaa ugcagggagt t 21

<210> SEQ ID NO 572
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hPTP1B1.4

<400> SEQUENCE: 572

caaugggaaa ugcagggag 19

<210> SEQ ID NO 573
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hPTP1B1.4

<400> SEQUENCE: 573

cucccugcau uucccauug 19

<210> SEQ ID NO 574
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hPTP1B1.4
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 574

caaugggaaa ugcagggagn n 21

<210> SEQ ID NO 575
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hPTP1B1.4
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 575

nncucccugc auuucccauu g 21

<210> SEQ ID NO 576
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hPTP1B1.5

<400> SEQUENCE: 576

-continued

```
ggaucagugg aaggagcuut c                                              21


<210> SEQ ID NO 577
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hPTP1B1.5

<400> SEQUENCE: 577

ggaucagugg aaggagcuu                                                 19


<210> SEQ ID NO 578
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hPTP1B1.5

<400> SEQUENCE: 578

aagcuccuuc cacugaucc                                                 19


<210> SEQ ID NO 579
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hPTP1B1.5
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 579

ggaucagugg aaggagcuun n                                              21


<210> SEQ ID NO 580
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hPTP1B1.5
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 580

nnaagcuccu uccacugauc c                                              21


<210> SEQ ID NO 581
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - mTCPTP1.1

<400> SEQUENCE: 581

guugucaugc uaaaccgaac t                                              21


<210> SEQ ID NO 582
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
```

-continued

```
<223> OTHER INFORMATION: Small interfering RNA - mTCPTP1.1

<400> SEQUENCE: 582

guugucaugc uaaaccgaa                                               19


<210> SEQ ID NO 583
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - mTCPTP1.1

<400> SEQUENCE: 583

uucgguuuag caugacaac                                               19


<210> SEQ ID NO 584
<211> LENGTH: 21
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - mTCPTP1.1
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 584

guugucaugc uaaaccgaan n                                            21


<210> SEQ ID NO 585
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - mTCPTP1.1
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 585

nnuucgguuu agcaugacaa c                                            21


<210> SEQ ID NO 586
<211> LENGTH: 21
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - mTCPTP1.2

<400> SEQUENCE: 586

cagaacagag ugaugguuga g                                            21


<210> SEQ ID NO 587
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - mTCPTP1.2

<400> SEQUENCE: 587

cagaacagag ugaugguug                                               19


<210> SEQ ID NO 588
```

-continued

<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - mTCPTP1.2

<400> SEQUENCE: 588

caaccaucac ucuguucug 19

<210> SEQ ID NO 589
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - mTCPTP1.2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 589

cagaacagag ugaugguugn n 21

<210> SEQ ID NO 590
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - mTCPTP1.2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 590

nncaaccauc acucuguucu g 21

<210> SEQ ID NO 591
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer (TC45 5' BamHI)

<400> SEQUENCE: 591

gggggatcc atgcccacca ccatcgagcg ggagtt 36

<210> SEQ ID NO 592
<211> LENGTH: 49
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide primer (TC45 3' EcoRI)

<400> SEQUENCE: 592

ggggaattct taggtgtctg tcaatcttgg cctttttctt tttcgttca 49

<210> SEQ ID NO 593
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hTCPTP1.4

<400> SEQUENCE: 593

-continued

guugucaugc ugaaccgcat t 21

<210> SEQ ID NO 594
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hTCPTP1.4

<400> SEQUENCE: 594

guugucaugc ugaaccgca 19

<210> SEQ ID NO 595
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hTCPTP1.4

<400> SEQUENCE: 595

ugcgguucag caugacaac 19

<210> SEQ ID NO 596
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hTCPTP1.4
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 596

guugucaugc ugaaccgcan n 21

<210> SEQ ID NO 597
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hTCPTP1.4
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 597

nnugcgguuc agcaugacaa c 21

<210> SEQ ID NO 598
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hTCPTP1.5

<400> SEQUENCE: 598

gcccauauga ucacagucgt g 21

<210> SEQ ID NO 599
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

-continued

<223> OTHER INFORMATION: Small interfering RNA - hTCPTP1.5

<400> SEQUENCE: 599

gcccauauga ucacagucg 19

<210> SEQ ID NO 600
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hTCPTP1.5

<400> SEQUENCE: 600

cgacugugau cauaugggc 19

<210> SEQ ID NO 601
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hTCPTP1.5
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 601

gcccauauga ucacagucgn n 21

<210> SEQ ID NO 602
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hTCPTP1.5
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 602

nncgacugug aucauauggg c 21

<210> SEQ ID NO 603
<211> LENGTH: 21
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hTCPTP1.6

<400> SEQUENCE: 603

ucgguuaaau gugcacagua c 21

<210> SEQ ID NO 604
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hTCPTP1.6

<400> SEQUENCE: 604

ucgguuaaau gugcacagu 19

<210> SEQ ID NO 605

-continued

<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hTCPTP1.6

<400> SEQUENCE: 605

acugugcaca uuuaaccga 19

<210> SEQ ID NO 606
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hTCPTP1.6
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 606

ucgguuaaau gugcacagun n 21

<210> SEQ ID NO 607
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hTCPTP1.6
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 607

nnacugugca cauuuaaccg a 21

<210> SEQ ID NO 608
<211> LENGTH: 21
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hTCPTP1.7

<400> SEQUENCE: 608

ugacuauccu cauagagugg g 21

<210> SEQ ID NO 609
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hTCPTP1.7

<400> SEQUENCE: 609

ugacuauccu cauagagug 19

<210> SEQ ID NO 610
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hTCPTP1.7

<400> SEQUENCE: 610

-continued

```
cacucuauga ggauaguca                                                    19


<210> SEQ ID NO 611
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hTCPTP1.7
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 611

ugacuauccu cauagagugn n                                                 21


<210> SEQ ID NO 612
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hTCPTP1.7
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 612

nncacucuau gaggauaguc a                                                 21


<210> SEQ ID NO 613
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hTCPTP1.1

<400> SEQUENCE: 613

agugagagaa ucuggcucct t                                                 21


<210> SEQ ID NO 614
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hTCPTP1.1

<400> SEQUENCE: 614

agugagagaa ucuggcucc                                                    19


<210> SEQ ID NO 615
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hTCPTP1.1

<400> SEQUENCE: 615

ggagccagau ucucucacu                                                    19


<210> SEQ ID NO 616
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
```

-continued

<223> OTHER INFORMATION: Small interfering RNA - hTCPTP1.1
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 616

agugagagaa ucuggcuccn n 21

<210> SEQ ID NO 617
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hTCPTP1.1
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 617

nnggagccag auucucucac u 21

<210> SEQ ID NO 618
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hTCPTP1.2

<400> SEQUENCE: 618

ggaagacuua ucuccugcct t 21

<210> SEQ ID NO 619
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hTCPTP1.2

<400> SEQUENCE: 619

ggaagacuua ucuccugcc 19

<210> SEQ ID NO 620
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hTCPTP1.2

<400> SEQUENCE: 620

ggcaggagau aagucuucc 19

<210> SEQ ID NO 621
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hTCPTP1.2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 621

-continued

ggaagacuua ucuccugccn n 21

<210> SEQ ID NO 622
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hTCPTP1.2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 622

nnggcaggag auaagucuuc c 21

<210> SEQ ID NO 623
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hTCPTP1.3

<400> SEQUENCE: 623

ggugaccgau guacaggact t 21

<210> SEQ ID NO 624
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hTCPTP1.3

<400> SEQUENCE: 624

ggugaccgau guacaggac 19

<210> SEQ ID NO 625
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hTCPTP1.3

<400> SEQUENCE: 625

guccuguaca ucggucacc 19

<210> SEQ ID NO 626
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - hTCPTP1.3
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 626

ggugaccgau guacaggacn n 21

<210> SEQ ID NO 627
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

-continued

<223> OTHER INFORMATION: Small interfering RNA - hTCPTP1.3
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 627

nnguccugua caucggucac c 21

<210> SEQ ID NO 628
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Hairpin vector - hPTP1B H1.2-HP4

<400> SEQUENCE: 628

tttgcccaaa ggagttacat tcgtaagaat gtaactcctt tgggcttttt 50

<210> SEQ ID NO 629
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Hairpin vector - hPTP1B H1.2-HP4

<400> SEQUENCE: 629

ctagaaaaag cccaaaggag ttacattctt acgaatgtaa ctcctttggg 50

<210> SEQ ID NO 630
<211> LENGTH: 50
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Hairpin vector - hPTP1B H1.2-HP4

<400> SEQUENCE: 630

uuugcccaaa ggaguuacau ucguaagaau guaacuccuu ugggcuuuuu 50

<210> SEQ ID NO 631
<211> LENGTH: 50
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Hairpin vector - hPTP1B H1.2-HP4

<400> SEQUENCE: 631

cuagaaaaag cccaaaggag uuacauucuu acgaauguaa cuccuuuggg 50

<210> SEQ ID NO 632
<211> LENGTH: 55
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Hairpin vector - hPTP1B H1.2-HP9

<400> SEQUENCE: 632

tttgcccaaa ggagttacat tccctgggta agaatgtaac tcctttgggc ttttt 55

<210> SEQ ID NO 633
<211> LENGTH: 55
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

-continued

<223> OTHER INFORMATION: Hairpin vector - hPTP1B H1.2-HP9

<400> SEQUENCE: 633

ctagaaaaag cccaaaggag ttacattctt acccagggaa tgtaactcct ttggg 55

<210> SEQ ID NO 634
<211> LENGTH: 55
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Hairpin vector - hPTP1B H1.2-HP9

<400> SEQUENCE: 634

uuugcccaaa ggaguuacau ucccugggua agaauguaac uccuuugggc uuuuu 55

<210> SEQ ID NO 635
<211> LENGTH: 55
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Hairpin vector - hPTP1B H1.2-HP9

<400> SEQUENCE: 635

cuagaaaaag cccaaaggag uuacauucuu acccagggaa uguaacuccu uuggg 55

<210> SEQ ID NO 636
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Hairpin vector - mPTP1B M1.1-HP4

<400> SEQUENCE: 636

tttgaagccc agaggagcta taagaatata gctcctctgg gcttcttttt 50

<210> SEQ ID NO 637
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Hairpin vector - mPTP1B M1.1-HP4

<400> SEQUENCE: 637

ctagaaaaag aagcccagag gagctatatt cttatagctc ctctgggctt 50

<210> SEQ ID NO 638
<211> LENGTH: 50
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Hairpin vector - mPTP1B M1.1-HP4

<400> SEQUENCE: 638

uuugaagccc agaggagcua uaagaauaua gcuccucugg gcuucuuuuu 50

<210> SEQ ID NO 639
<211> LENGTH: 50
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Hairpin vector - mPTP1B M1.1-HP4

<400> SEQUENCE: 639

-continued

cuagaaaaag aagcccagag gagcuauauu cuuauagcuc cucugggcuu 50

<210> SEQ ID NO 640
<211> LENGTH: 55
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Hairpin vector - mPTP1B M1.1-HP9

<400> SEQUENCE: 640

tttgaagccc agaggagcta tagggtgaga atatagctcc tctgggcttc ttttt 55

<210> SEQ ID NO 641
<211> LENGTH: 55
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Hairpin vector - mPTP1B M1.1-HP9

<400> SEQUENCE: 641

ctagaaaaag aagcccagag gagctatatt ctcaccctat agctcctctg ggctt 55

<210> SEQ ID NO 642
<211> LENGTH: 55
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Hairpin vector - mPTP1B M1.1-HP9

<400> SEQUENCE: 642

uuugaagccc agaggagcua uagggugaga auauagcucc ucugggcuuc uuuuu 55

<210> SEQ ID NO 643
<211> LENGTH: 55
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Hairpin vector - mPTP1B M1.1-HP9

<400> SEQUENCE: 643

cuagaaaaag aagcccagag gagcuauauu cucacccuau agcuccucug ggcuu 55

<210> SEQ ID NO 644
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide selected from scanning open
reading frame of TC45 mRNA

<400> SEQUENCE: 644

aacagauaca gagauguaag c 21

<210> SEQ ID NO 645
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Oligonucleotide selected from scanning open
reading frame of TC45 mRNA

<400> SEQUENCE: 645

aagcccauau gaucacaguc g 21

-continued

```
<210> SEQ ID NO 646
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - DSP3.4

<400> SEQUENCE: 646

ggugacacau auucugucut t                                              21


<210> SEQ ID NO 647
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - DSP3.4

<400> SEQUENCE: 647

ggugacacau auucugucu                                                 19


<210> SEQ ID NO 648
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - DSP3.4

<400> SEQUENCE: 648

agacagaaua ugugucacc                                                 19


<210> SEQ ID NO 649
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - DSP3.4
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 649

ggugacacau auucugucun n                                              21


<210> SEQ ID NO 650
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - DSP3.4
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 650

nnagacagaa uaugugucac c                                              21


<210> SEQ ID NO 651
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - DHFR.1
```

-continued

<400> SEQUENCE: 651

gaccugguuc uccauuccut t 21

<210> SEQ ID NO 652
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - DHFR.1

<400> SEQUENCE: 652

gaccugguuc uccauuccu 19

<210> SEQ ID NO 653
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - DHFR.1

<400> SEQUENCE: 653

aggaauggag aaccagguc 19

<210> SEQ ID NO 654
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - DHFR.1
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 654

gaccugguuc uccauuccun n 21

<210> SEQ ID NO 655
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - DHFR.1
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 655

nnaggaaugg agaaccaggu c 21

<210> SEQ ID NO 656
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - DHFR.3

<400> SEQUENCE: 656

gcaguguauu ugcuagguct t 21

<210> SEQ ID NO 657
<211> LENGTH: 19
<212> TYPE: RNA

-continued

<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - DHFR.3

<400> SEQUENCE: 657

gcaguguauu ugcuagguc 19

<210> SEQ ID NO 658
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - DHFR.3

<400> SEQUENCE: 658

gaccuagcaa auacacugc 19

<210> SEQ ID NO 659
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - DHFR.3
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 659

gcaguguauu ugcuaggucn n 21

<210> SEQ ID NO 660
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - DHFR.3
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 660

nngaccuagc aaauacacug c 21

<210> SEQ ID NO 661
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - DHFR.4

<400> SEQUENCE: 661

gucagcgagc agguucucat t 21

<210> SEQ ID NO 662
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - DHFR.4

<400> SEQUENCE: 662

gucagcgagc agguucuca 19

-continued

<210> SEQ ID NO 663
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - DHFR.4

<400> SEQUENCE: 663

ugagaaccug cucgcugac 19

<210> SEQ ID NO 664
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - DHFR.4
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 664

gucagcgagc agguucucan n 21

<210> SEQ ID NO 665
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - DHFR.4
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 665

nnugagaacc ugcucgcuga c 21

<210> SEQ ID NO 666
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - TYMS.1

<400> SEQUENCE: 666

ccaaacgugu guucuggaat t 21

<210> SEQ ID NO 667
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - TYMS.1

<400> SEQUENCE: 667

ccaaacgugu guucuggaa 19

<210> SEQ ID NO 668
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - TYMS.1

-continued

<400> SEQUENCE: 668

uuccagaaca cacguuugg 19

<210> SEQ ID NO 669
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - TYMS.1
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 669

ccaaacgugu guucuggaan n 21

<210> SEQ ID NO 670
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - TYMS.1
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 670

nnuuccagaa cacacguuug g 21

<210> SEQ ID NO 671
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - TYMS.2

<400> SEQUENCE: 671

ccaacccuga cgacagaagt t 21

<210> SEQ ID NO 672
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - TYMS.2

<400> SEQUENCE: 672

ccaacccuga cgacagaag 19

<210> SEQ ID NO 673
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - TYMS.2

<400> SEQUENCE: 673

cuucugucgu cagguugg 19

<210> SEQ ID NO 674
<211> LENGTH: 21
<212> TYPE: DNA

-continued

<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - TYMS.2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 674

ccaacccuga cgacagaagn n 21

<210> SEQ ID NO 675
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - TYMS.2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 675

nncuucuguc gucaggguug g 21

<210> SEQ ID NO 676
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - TYMS.3

<400> SEQUENCE: 676

gccaggugac uuuauacact t 21

<210> SEQ ID NO 677
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - TYMS.3

<400> SEQUENCE: 677

gccaggugac uuuauacac 19

<210> SEQ ID NO 678
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - TYMS.3

<400> SEQUENCE: 678

guguauaaag ucaccuggc 19

<210> SEQ ID NO 679
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - TYMS.3
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

-continued

<400> SEQUENCE: 679

gccaggugac uuuauacacn n 21

<210> SEQ ID NO 680
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - TYMS.3
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 680

nnguguauaa agucaccugg c 21

<210> SEQ ID NO 681
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - TYMS.4

<400> SEQUENCE: 681

cccagaccuu ucccaaagct t 21

<210> SEQ ID NO 682
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - TYMS.4

<400> SEQUENCE: 682

cccagaccuu ucccaaagc 19

<210> SEQ ID NO 683
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - TYMS.4

<400> SEQUENCE: 683

gcuuugggaa aggucuggg 19

<210> SEQ ID NO 684
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - TYMS.4
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 684

cccagaccuu ucccaaagcn n 21

<210> SEQ ID NO 685
<211> LENGTH: 21
<212> TYPE: DNA

-continued

<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - TYMS.4
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 685

nngcuuuggg aaaggucugg g 21

<210> SEQ ID NO 686
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - TOP1.1

<400> SEQUENCE: 686

gauagagccu ccuggacuut t 21

<210> SEQ ID NO 687
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - TOP1.1

<400> SEQUENCE: 687

gauagagccu ccuggacuu 19

<210> SEQ ID NO 688
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - TOP1.1

<400> SEQUENCE: 688

aaguccagga ggcucuauc 19

<210> SEQ ID NO 689
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - TOP1.1
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 689

gauagagccu ccuggacuun n 21

<210> SEQ ID NO 690
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - TOP1.1
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

-continued

<400> SEQUENCE: 690

nnaaguccag gaggcucuau c 21

<210> SEQ ID NO 691
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - TOP1.2

<400> SEQUENCE: 691

guccggcaug auaacaaggt t 21

<210> SEQ ID NO 692
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - TOP1.2

<400> SEQUENCE: 692

guccggcaug auaacaagg 19

<210> SEQ ID NO 693
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - TOP1.2

<400> SEQUENCE: 693

ccuuguuauc augccggac 19

<210> SEQ ID NO 694
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - TOP1.2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 694

guccggcaug auaacaaggn n 21

<210> SEQ ID NO 695
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - TOP1.2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 695

nnccuuguua ucaugccgga c 21

<210> SEQ ID NO 696
<211> LENGTH: 21
<212> TYPE: DNA

-continued

```
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - TOP1.3

<400> SEQUENCE: 696

ggagaaacag cggacacugt t                                              21


<210> SEQ ID NO 697
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - TOP1.3

<400> SEQUENCE: 697

ggagaaacag cggacacug                                                 19


<210> SEQ ID NO 698
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - TOP1.3

<400> SEQUENCE: 698

caguguccgc uguuucucc                                                 19


<210> SEQ ID NO 699
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - TOP1.3
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 699

ggagaaacag cggacacugn n                                              21


<210> SEQ ID NO 700
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - TOP1.3
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 700

nncaguguccc gcuguuucuc c                                              21


<210> SEQ ID NO 701
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - TOP1.4

<400> SEQUENCE: 701

gcagcccgag gaugaucuut t                                              21
```

-continued

<210> SEQ ID NO 702
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - TOP1.4

<400> SEQUENCE: 702

gcagcccgag gaugaucuu 19

<210> SEQ ID NO 703
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - TOP1.4

<400> SEQUENCE: 703

aagaucaucc ucgggcugc 19

<210> SEQ ID NO 704
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - TOP1.4
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 704

gcagcccgag gaugaucuun n 21

<210> SEQ ID NO 705
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - TOP1.4
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 705

nnaagaucau ccucgggcug c 21

<210> SEQ ID NO 706
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - IKK.1

<400> SEQUENCE: 706

gagucuccuc uggggaagct t 21

<210> SEQ ID NO 707
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - IKK.1

-continued

<400> SEQUENCE: 707

gagucuccuc uggggaagc 19

<210> SEQ ID NO 708
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - IKK.1

<400> SEQUENCE: 708

gcuuccccag aggagacuc 19

<210> SEQ ID NO 709
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - IKK.1
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 709

gagucuccuc uggggaagcn n 21

<210> SEQ ID NO 710
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - IKK.1
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 710

nngcuucccc agaggagacu c 21

<210> SEQ ID NO 711
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - IKK.2

<400> SEQUENCE: 711

ggaguuccuc augugcaagt t 21

<210> SEQ ID NO 712
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - IKK.2

<400> SEQUENCE: 712

ggaguuccuc augugcaag 19

<210> SEQ ID NO 713
<211> LENGTH: 19
<212> TYPE: RNA

-continued

<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - IKK.2

<400> SEQUENCE: 713

cuugcacaug aggaacucc 19

<210> SEQ ID NO 714
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - IKK.2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 714

ggaguuccuc augugcaagn n 21

<210> SEQ ID NO 715
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - IKK.2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 715

nncuugcaca ugaggaacuc c 21

<210> SEQ ID NO 716
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - IKK.3

<400> SEQUENCE: 716

ggccucugug aaagcccagt t 21

<210> SEQ ID NO 717
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - IKK.3

<400> SEQUENCE: 717

ggccucugug aaagcccag 19

<210> SEQ ID NO 718
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - IKK.3

<400> SEQUENCE: 718

cugggcuuuc acagaggcc 19

-continued

<210> SEQ ID NO 719
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - IKK.3
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 719

ggccucugug aaagcccagn n 21

<210> SEQ ID NO 720
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - IKK.3
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 720

nncugggcuu ucacagaggc c 21

<210> SEQ ID NO 721
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - IKK.4

<400> SEQUENCE: 721

cacgcugcuc uugauguggt t 21

<210> SEQ ID NO 722
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - IKK.4

<400> SEQUENCE: 722

cacgcugcuc uugaugugg 19

<210> SEQ ID NO 723
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - IKK.4

<400> SEQUENCE: 723

ccacaucaag agcagcgug 19

<210> SEQ ID NO 724
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - IKK.4
<220> FEATURE:

-continued

<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 724

cacgcugcuc uugauguggn n 21

<210> SEQ ID NO 725
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - IKK.4
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 725

nnccacauca agagcagcgu g 21

<210> SEQ ID NO 726
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - MKK4.1

<400> SEQUENCE: 726

gugggcaaau aauggcagut t 21

<210> SEQ ID NO 727
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - MKK4.1

<400> SEQUENCE: 727

gugggcaaau aauggcagu 19

<210> SEQ ID NO 728
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - MKK4.1

<400> SEQUENCE: 728

acugccauua uuugcccac 19

<210> SEQ ID NO 729
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - MKK4.1
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 729

gugggcaaau aauggcagun n 21

-continued

```
<210> SEQ ID NO 730
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - MKK4.1
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 730

nnacugccau uauuugccca c                                                21


<210> SEQ ID NO 731
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - MKK4.2

<400> SEQUENCE: 731

cugugaaagc acuaaaccat t                                                21


<210> SEQ ID NO 732
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - MKK4.2

<400> SEQUENCE: 732

cugugaaagc acuaaacca                                                   19


<210> SEQ ID NO 733
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - MKK4.2

<400> SEQUENCE: 733

ugguuuagug cuuucacag                                                   19


<210> SEQ ID NO 734
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - MKK4.2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 734

cugugaaagc acuaaaccan n                                                21


<210> SEQ ID NO 735
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - MKK4.2
<220> FEATURE:
```

-continued

<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 735

nnugguuuag ugcuuucaca g 21

<210> SEQ ID NO 736
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - MKK4.3

<400> SEQUENCE: 736

ggagauccuc cgcagcugat t 21

<210> SEQ ID NO 737
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - MKK4.3

<400> SEQUENCE: 737

ggagauccuc cgcagcuga 19

<210> SEQ ID NO 738
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - MKK4.3

<400> SEQUENCE: 738

ucagcugcgg aggaucucc 19

<210> SEQ ID NO 739
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - MKK4.3
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 739

ggagauccuc cgcagcugan n 21

<210> SEQ ID NO 740
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - MKK4.3
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 740

nnucagcugc ggaggaucuc c 21

-continued

<210> SEQ ID NO 741
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - MKK4.4

<400> SEQUENCE: 741

gcucuuuaua cuuuggccut t 21

<210> SEQ ID NO 742
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - MKK4.4

<400> SEQUENCE: 742

gcucuuuaua cuuuggccu 19

<210> SEQ ID NO 743
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - MKK4.4

<400> SEQUENCE: 743

aggccaaagu auaaagagc 19

<210> SEQ ID NO 744
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - MKK4.4
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 744

gcucuuuaua cuuuggccun n 21

<210> SEQ ID NO 745
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - MKK4.4
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 745

nnaggccaaa guauaaagag c 21

<210> SEQ ID NO 746
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - MKK7.1

-continued

<400> SEQUENCE: 746

gcagacgggc uaccugacct t 21

<210> SEQ ID NO 747
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - MKK7.1

<400> SEQUENCE: 747

gcagacgggc uaccugacc 19

<210> SEQ ID NO 748
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - MKK7.1

<400> SEQUENCE: 748

ggucagguag cccgucugc 19

<210> SEQ ID NO 749
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - MKK7.1
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 749

gcagacgggc uaccugaccn n 21

<210> SEQ ID NO 750
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - MKK7.1
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 750

nnggucaggu agcccgucug c 21

<210> SEQ ID NO 751
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - MKK7.2

<400> SEQUENCE: 751

cacggacguc uucaucgcct t 21

<210> SEQ ID NO 752
<211> LENGTH: 19
<212> TYPE: RNA

-continued

<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - MKK7.2

<400> SEQUENCE: 752

cacggacguc uucaucgcc 19

<210> SEQ ID NO 753
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - MKK7.2

<400> SEQUENCE: 753

ggcgaugaag acguccgug 19

<210> SEQ ID NO 754
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - MKK7.2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 754

cacggacguc uucaucgccn n 21

<210> SEQ ID NO 755
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - MKK7.2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 755

nnggcgauga agacguccgu g 21

<210> SEQ ID NO 756
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - MKK7.3

<400> SEQUENCE: 756

gaagcggaug cagggcccct t 21

<210> SEQ ID NO 757
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - MKK7.3

<400> SEQUENCE: 757

gaagcggaug cagggcccc 19

-continued

<210> SEQ ID NO 758
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - MKK7.3

<400> SEQUENCE: 758

ggggcccugc auccgcuuc 19

<210> SEQ ID NO 759
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - MKK7.3
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 759

gaagcggaug cagggccccn n 21

<210> SEQ ID NO 760
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - MKK7.3
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 760

nnggggcccu gcauccgcuu c 21

<210> SEQ ID NO 761
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - MKK7.4

<400> SEQUENCE: 761

cugcaagacg gacuuugagt t 21

<210> SEQ ID NO 762
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - MKK7.4

<400> SEQUENCE: 762

cugcaagacg gacuuugag 19

<210> SEQ ID NO 763
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - MKK7.4

-continued

<400> SEQUENCE: 763

cucaaagucc gucuugcag 19

<210> SEQ ID NO 764
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - MKK7.4
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 764

cugcaagacg gacuuugagn n 21

<210> SEQ ID NO 765
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - MKK7.4
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 765

nncucaaagu ccgucuugca g 21

<210> SEQ ID NO 766
<211> LENGTH: 55
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Hairpin Vector - HP53-HP9

<400> SEQUENCE: 766

tttgactcca gtggtaatct acttcaagag agtagattac cactggagtc ttttt 55

<210> SEQ ID NO 767
<211> LENGTH: 55
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Hairpin Vector - HP53-HP9

<400> SEQUENCE: 767

ctagaaaaag actccagtgg taatctactc tcttgaagta gattaccact ggagt 55

<210> SEQ ID NO 768
<211> LENGTH: 55
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Hairpin Vector - HP53-HP9

<400> SEQUENCE: 768

uuugacucca gugguaaucu acuucaagag aguagauuac cacuggaguc uuuuu 55

<210> SEQ ID NO 769
<211> LENGTH: 55
<212> TYPE: RNA

-continued

<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Hairpin Vector - HP53-HP9

<400> SEQUENCE: 769

cuagaaaaag acuccagugg uaaucuacuc ucuugaagua gauuaccacu ggagu 55

<210> SEQ ID NO 770
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - TCPTP1

<400> SEQUENCE: 770

aacagauaca gagauguaa 19

<210> SEQ ID NO 771
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - TCPTP1

<400> SEQUENCE: 771

uuacaucucu guaucuguu 19

<210> SEQ ID NO 772
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - TCPTP1
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 772

aacagauaca gagauguaan n 21

<210> SEQ ID NO 773
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - TCPTP1
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 773

nnuuacaucu cuguaucugu u 21

<210> SEQ ID NO 774
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - TCPTP2

<400> SEQUENCE: 774

aagcccauau gaucacagu 19

-continued

<210> SEQ ID NO 775
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - TCPTP2

<400> SEQUENCE: 775

acugugauca uaugggcuu 19

<210> SEQ ID NO 776
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - TCPTP2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 20, 21
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 776

aagcccauau gaucacagun n 21

<210> SEQ ID NO 777
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interefering RNA - TCPTP2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 1, 2
<223> OTHER INFORMATION: n = A,T,C,G or U

<400> SEQUENCE: 777

nnacugugau cauaugggcu u 21

<210> SEQ ID NO 778
<211> LENGTH: 926
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 778

ccccgccgct cctcctccct gtaacatgcc atagtgcgcc tgcgaccaca cggccggggc 60

gctagcgttc gccttcagcc accatgggga atgggatgaa caagatcctg cccggcctgt 120

acatcggcaa cttcaaagat gccagagacg cggaacaatt gagcaagaac aaggtgacac 180

atattctgtc tgtccacgat agtgccaggc ctatgttgga gggagttaaa tacctgtgca 240

tcccagcagc ggattcacca tctcaaaacc tgacaagaca tttcaaagaa agtattaaat 300

tcattcacga gtgccggctc cgcggtgaga gctgccttgt acactgcctg gccggggtct 360

ccaggagcgt gacactggtg atcgcataca tcatgaccgt cactgacttt ggctgggagg 420

atgccctgca caccgtgcgt gctgggagat cctgtgccaa ccccaacgtg ggcttccaga 480

gacagctcca ggagtttgag aagcatgagg tccatcagta tcggcagtgg ctgaaggaag 540

aatatggaga gagccctttg caggatgcag aagaagccaa aaacattctg gccgctccag 600

gaattctgaa gttctgggcc tttctcagaa gactgtaatg tacctgaagt ttctgaaata 660

ttgcaaaccc gcagagttta ggctggtgct gccaaaaaga aaagcaacat agagtttaag 720

-continued

```
tatccagtag tgatttgtaa acttgttttt catttgaagc tgaatatata cgtagtcatg     780

tttatgttga gaactaagga tattctttag caagagaaaa tattttcccc ttatccccac     840

tgctgtggag gtttctgtac ctcgcttgga tgcctgtaag gatcccggga gccttgccgc     900

actgccttgt gggtggcttg gcgctc                                          926


<210> SEQ ID NO 779
<211> LENGTH: 184
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 779

Met Gly Asn Gly Met Asn Lys Ile Leu Pro Gly Leu Tyr Ile Gly Asn
1               5                   10                  15

Phe Lys Asp Ala Arg Asp Ala Glu Gln Leu Ser Lys Asn Lys Val Thr
            20                  25                  30

His Ile Leu Ser Val His Asp Ser Ala Arg Pro Met Leu Glu Gly Val
        35                  40                  45

Lys Tyr Leu Cys Ile Pro Ala Ala Asp Ser Pro Ser Gln Asn Leu Thr
    50                  55                  60

Arg His Phe Lys Glu Ser Ile Lys Phe Ile His Glu Cys Arg Leu Arg
65                  70                  75                  80

Gly Glu Ser Cys Leu Val His Cys Leu Ala Gly Val Ser Arg Ser Val
                85                  90                  95

Thr Leu Val Ile Ala Tyr Ile Met Thr Val Thr Asp Phe Gly Trp Glu
            100                 105                 110

Asp Ala Leu His Thr Val Arg Ala Gly Arg Ser Cys Ala Asn Pro Asn
        115                 120                 125

Val Gly Phe Gln Arg Gln Leu Gln Glu Phe Glu Lys His Glu Val His
    130                 135                 140

Gln Tyr Arg Gln Trp Leu Lys Glu Glu Tyr Gly Glu Ser Pro Leu Gln
145                 150                 155                 160

Asp Ala Glu Glu Ala Lys Asn Ile Leu Ala Ala Pro Gly Ile Leu Lys
                165                 170                 175

Phe Trp Ala Phe Leu Arg Arg Leu
            180


<210> SEQ ID NO 780
<211> LENGTH: 707
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 780

tgacccgctg tcctgtgccc tttcccagcg atgggcgtgc agccccccaa cttctcctgg      60

gtgcttccgg gccggctggc gggactggcg ctgccgcggc tccccgccca ctaccagttc     120

ctgttggacc tgggcgtgcg gcacctggtg tccctgacgg agcgcgggcc ccctcacagc     180

gacagctgcc ccggcctcac cctgcaccgc ctgcgcatcc ccgacttctg cccgccggcc     240

cccgaccaga tcgaccgctt cgtgcagatc gtggacgagg ccaacgcacg gggagaggct     300

gtgggagtgc actgtgctct gggctttggc cgcactggca ccatgctggc ctgttacctg     360

gtgaaggagc ggggcttggc tgcaggagat gccattgctg aaatccgacg actacgaccc     420

ggctccatcg agacctatga gcaggagaaa gcagtcttcc agttctacca gcgaacgaaa     480
```

-continued

```
taaggggcct tagtaccctt ctaccaggcc ctcactcccc ttccccatgt tgtcgatggg    540

gccagagatg aagggaagtg gactaaagta ttaaaccctc tagctcccat tggctgaaga    600

cactgaagta gcccacccct gcaggcaggt cctgattgaa ggggaggctt gtactgcttt    660

gttgaataaa tgagttttac gaaccaaaaa aaaaaaaaaa aaaaaaa                  707


<210> SEQ ID NO 781
<211> LENGTH: 150
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 781

Met Gly Val Gln Pro Pro Asn Phe Ser Trp Val Leu Pro Gly Arg Leu
1               5                   10                  15

Ala Gly Leu Ala Leu Pro Arg Leu Pro Ala His Tyr Gln Phe Leu Leu
            20                  25                  30

Asp Leu Gly Val Arg His Leu Val Ser Leu Thr Glu Arg Gly Pro Pro
        35                  40                  45

His Ser Asp Ser Cys Pro Gly Leu Thr Leu His Arg Leu Arg Ile Pro
    50                  55                  60

Asp Phe Cys Pro Pro Ala Pro Asp Gln Ile Asp Arg Phe Val Gln Ile
65                  70                  75                  80

Val Asp Glu Ala Asn Ala Arg Gly Glu Ala Val Gly Val His Cys Ala
                85                  90                  95

Leu Gly Phe Gly Arg Thr Gly Thr Met Leu Ala Cys Tyr Leu Val Lys
            100                 105                 110

Glu Arg Gly Leu Ala Ala Gly Asp Ala Ile Ala Glu Ile Arg Arg Leu
        115                 120                 125

Arg Pro Gly Ser Ile Glu Thr Tyr Glu Gln Glu Lys Ala Val Phe Gln
    130                 135                 140

Phe Tyr Gln Arg Thr Lys
145                 150


<210> SEQ ID NO 782
<211> LENGTH: 833
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 782

ggcccccgt tccccgccag gctgcaggcg tcgggcctgg gccgtcaggg cagctgtgac      60

cggatcgctt cccgggcggc gagctggggg tgcacccgga ccgccgcccc cgggatcatg    120

ggcaatggca tgaccaaggt acttcctgga ctctacctcg gaaacttcat tgatgccaaa    180

gacctggatc agctgggccg aaataagatc acacacatca tctctatcca tgagtcaccc    240

cagcctctgc tgcaggatat cacctacctt cgcatcccgg tcgctgatac ccctgaggta    300

cccatcaaaa agcacttcaa agaatgtatc aacttcatcc actgctgccg ccttaatggg    360

gggaactgcc ttgtgcactg ctttgcaggc atctctcgca gcaccacgat tgtgacagcg    420

tatgtgatga ctgtgacggg gctaggctgg cgggacgtgc ttgaagccat caaggccacc    480

aggcccatcg ccaaccccaa cccaggcttt aggcagcagc ttgaagagtt tggctgggcc    540

agttcccaga agcttcgccg gcagctggag gagcgcttcg gcgagagccc cttccgcgac    600

gaggaggagt tgcgcgcgct gctgccgctg tgcaagcgct gccggcaggg ctccgcgacc    660
```

-continued

```
tcggcctcct ccgccgggcc gcactcagca gcctccgagg gaaccgtgca gcgcctggtg    720

ccgcgcacgc cccgggaagc ccaccggccg ctgccgctgc tggcgcgcgt caagcagact    780

ttctcttgcc tcccccggtg tctgtcccgc aagggcggca agtgaggatg cag           833


<210> SEQ ID NO 783
<211> LENGTH: 235
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 783

Met Gly Asn Gly Met Thr Lys Val Leu Pro Gly Leu Tyr Leu Gly Asn
1               5                   10                  15

Phe Ile Asp Ala Lys Asp Leu Asp Gln Leu Gly Arg Asn Lys Ile Thr
            20                  25                  30

His Ile Ile Ser Ile His Glu Ser Pro Gln Pro Leu Leu Gln Asp Ile
        35                  40                  45

Thr Tyr Leu Arg Ile Pro Val Ala Asp Thr Pro Glu Val Pro Ile Lys
    50                  55                  60

Lys His Phe Lys Glu Cys Ile Asn Phe Ile His Cys Cys Arg Leu Asn
65                  70                  75                  80

Gly Gly Asn Cys Leu Val His Cys Phe Ala Gly Ile Ser Arg Ser Thr
                85                  90                  95

Thr Ile Val Thr Ala Tyr Val Met Thr Val Thr Gly Leu Gly Trp Arg
            100                 105                 110

Asp Val Leu Glu Ala Ile Lys Ala Thr Arg Pro Ile Ala Asn Pro Asn
        115                 120                 125

Pro Gly Phe Arg Gln Gln Leu Glu Glu Phe Gly Trp Ala Ser Ser Gln
    130                 135                 140

Lys Leu Arg Arg Gln Leu Glu Glu Arg Phe Gly Glu Ser Pro Phe Arg
145                 150                 155                 160

Asp Glu Glu Glu Leu Arg Ala Leu Leu Pro Leu Cys Lys Arg Cys Arg
                165                 170                 175

Gln Gly Ser Ala Thr Ser Ala Ser Ser Ala Gly Pro His Ser Ala Ala
            180                 185                 190

Ser Glu Gly Thr Val Gln Arg Leu Val Pro Arg Thr Pro Arg Glu Ala
        195                 200                 205

His Arg Pro Leu Pro Leu Leu Ala Arg Val Lys Gln Thr Phe Ser Cys
    210                 215                 220

Leu Pro Arg Cys Leu Ser Arg Lys Gly Gly Lys
225                 230                 235


<210> SEQ ID NO 784
<211> LENGTH: 1711
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 784

cctgggaaga agttatctat ctctcgagtg acattcaaga tataccgtac ccctcggttc     60

tgtaagtcct ctaagttgga ggcattccat tctgagccgg ccccatgacc ctgagcacgt    120

tggcccgcaa gaggaaggcg cccctcgctt gcacctgcag cctcggtggc cccgacatga    180

ttccttactt ctccgccaac gcggtcatct cgcagaacgc catcaaccag ctcatcagcg    240

agagctttct aactgtcaaa ggtgctgccc tttttctacc acggggaaat ggctcatcca    300
```

-continued

```
caccaagaat cagccacaga cggaacaagc atgcaggcga tctccaacag catctccaag    360
caatgttcat tttactccgc ccagaagaca acatcaggct ggctgtaaga ctggaaagta    420
cttaccagaa tcgaacacgc tatatggtag tggtttcaac taatggtaga caagacactg    480
aagaaagcat cgtcctagga atggatttct cctctaatga cagtagcact tgtaccatgg    540
gcttagtttt gcctctctgg agcgacacgc taattcattt ggatggtgat ggtgggttca    600
gtgtatcgac ggataacaga gttcacatat tcaaacctgt atctgtgcag gcaatgtggt    660
ctgcactaca gagcttacac aaggcttgtg aagtcgccag agcgcataac tactacccag    720
gcagcctatt tctcacttgg gtgagttatt atgagagcca tatcaactca gatcaatcct    780
cagtcaatga atggaatgca atgcaagatg tacagtccca ccggcccgac tctccagctc    840
tcttcaccga catacctact gaacgtgaac gaacagaaag gctaattaaa accaaattaa    900
gggagatcat gatgcagaag gatttggaga atattacatc caaagagata agaacagagt    960
tggaaatgca aatggtgtgc aacttgcggg aattcaagga atttatagac aatgaaatga   1020
tagtgatcct tggtcaaatg gatagcccta cacagatatt tgagcatgtg ttcctgggct   1080
cagaatggaa tgcctccaac ttagaggact tacagaaccg agggtacgg tatatcttga    1140
atgtcactcg agagatagat aacttcttcc caggagtctt tgagtatcat aacattcggg   1200
tatatgatga agaggcaacg gatctcctgg cgtactggaa tgacacttac aaattcatct   1260
ctaaagcaaa gaaacatgga tctaaatgcc ttgtgcactg caaaatgggg gtgagtcgct   1320
cagcctccac cgtgattgcc tatgcaatga aggaatatgg ctggaatctg gaccgagcct   1380
atgactatgt gaaagaaaga cgaacggtaa ccaagcccaa cccaagcttc atgagacaac   1440
tggaagagta tcaggggatc ttgctggcaa gcttcctagg cttgattcat ggagggaggg   1500
acaagccctg gggagagaaa agcacagaat ttgagtcagt agatctggtt tccattcctg   1560
gttcaccctc ttgctgcaac cctgagaagt tacttcacat ttctcatcct tacctgaccc   1620
catctataaa atgaaaatca agagatccat ctcacagggt tattgtgaat aaaaatgtgt   1680
ttgaatgttt ataaaaaaaa aaaaaaaaaa a                                  1711
```

```
<210> SEQ ID NO 785
<211> LENGTH: 509
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 785

Met Thr Leu Ser Thr Leu Ala Arg Lys Arg Lys Ala Pro Leu Ala Cys
1               5                   10                  15

Thr Cys Ser Leu Gly Gly Pro Asp Met Ile Pro Tyr Phe Ser Ala Asn
            20                  25                  30

Ala Val Ile Ser Gln Asn Ala Ile Asn Gln Leu Ile Ser Glu Ser Phe
        35                  40                  45

Leu Thr Val Lys Gly Ala Ala Leu Phe Leu Pro Arg Gly Asn Gly Ser
    50                  55                  60

Ser Thr Pro Arg Ile Ser His Arg Arg Asn Lys His Ala Gly Asp Leu
65                  70                  75                  80

Gln Gln His Leu Gln Ala Met Phe Ile Leu Leu Arg Pro Glu Asp Asn
                85                  90                  95

Ile Arg Leu Ala Val Arg Leu Glu Ser Thr Tyr Gln Asn Arg Thr Arg
```

-continued

```
            100                 105                 110

Tyr Met Val Val Val Ser Thr Asn Gly Arg Gln Asp Thr Glu Glu Ser
        115                 120                 125

Ile Val Leu Gly Met Asp Phe Ser Ser Asn Asp Ser Ser Thr Cys Thr
    130                 135                 140

Met Gly Leu Val Leu Pro Leu Trp Ser Asp Thr Leu Ile His Leu Asp
145                 150                 155                 160

Gly Asp Gly Gly Phe Ser Val Ser Thr Asp Asn Arg Val His Ile Phe
                165                 170                 175

Lys Pro Val Ser Val Gln Ala Met Trp Ser Ala Leu Gln Ser Leu His
            180                 185                 190

Lys Ala Cys Glu Val Ala Arg Ala His Asn Tyr Tyr Pro Gly Ser Leu
        195                 200                 205

Phe Leu Thr Trp Val Ser Tyr Tyr Glu Ser His Ile Asn Ser Asp Gln
    210                 215                 220

Ser Ser Val Asn Glu Trp Asn Ala Met Gln Asp Val Gln Ser His Arg
225                 230                 235                 240

Pro Asp Ser Pro Ala Leu Phe Thr Asp Ile Pro Thr Glu Arg Glu Arg
                245                 250                 255

Thr Glu Arg Leu Ile Lys Thr Lys Leu Arg Glu Ile Met Met Gln Lys
            260                 265                 270

Asp Leu Glu Asn Ile Thr Ser Lys Glu Ile Arg Thr Glu Leu Glu Met
        275                 280                 285

Gln Met Val Cys Asn Leu Arg Glu Phe Lys Glu Phe Ile Asp Asn Glu
    290                 295                 300

Met Ile Val Ile Leu Gly Gln Met Asp Ser Pro Thr Gln Ile Phe Glu
305                 310                 315                 320

His Val Phe Leu Gly Ser Glu Trp Asn Ala Ser Asn Leu Glu Asp Leu
                325                 330                 335

Gln Asn Arg Gly Val Arg Tyr Ile Leu Asn Val Thr Arg Glu Ile Asp
            340                 345                 350

Asn Phe Phe Pro Gly Val Phe Glu Tyr His Asn Ile Arg Val Tyr Asp
        355                 360                 365

Glu Glu Ala Thr Asp Leu Leu Ala Tyr Trp Asn Asp Thr Tyr Lys Phe
    370                 375                 380

Ile Ser Lys Ala Lys Lys His Gly Ser Lys Cys Leu Val His Cys Lys
385                 390                 395                 400

Met Gly Val Ser Arg Ser Ala Ser Thr Val Ile Ala Tyr Ala Met Lys
                405                 410                 415

Glu Tyr Gly Trp Asn Leu Asp Arg Ala Tyr Asp Tyr Val Lys Glu Arg
            420                 425                 430

Arg Thr Val Thr Lys Pro Asn Pro Ser Phe Met Arg Gln Leu Glu Glu
        435                 440                 445

Tyr Gln Gly Ile Leu Leu Ala Ser Phe Leu Gly Leu Ile His Gly Gly
    450                 455                 460

Arg Asp Lys Pro Trp Gly Glu Lys Ser Thr Glu Phe Glu Ser Val Asp
465                 470                 475                 480

Leu Val Ser Ile Pro Gly Ser Pro Ser Cys Cys Asn Pro Glu Lys Leu
                485                 490                 495

Leu His Ile Ser His Pro Tyr Leu Thr Pro Ser Ile Lys
            500                 505
```

-continued

<210> SEQ ID NO 786
<211> LENGTH: 1165
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 786

```
ggccagtggg ggtggctggg cgtgcggctg ctacatgccc cacggaccag aacctcccga     60
cgcggccagg ccccggcaca cccagctgca gaaaggagag aaaatccctt ggctctaaaa    120
tgacatctgg agaagtgaag acaagcctca agaatgccta ctcatctgcc aagaggctgt    180
cgccgaagat ggaggaggaa ggggaggagg aggactactg cacccctgga gcctttgagc    240
tggagcggct cttctggaag ggcagtcccc agtacaccca cgtcaacgag gtctggccca    300
agctctacat tggcgatgag gcgacggcgc tggaccgcta taggctgcag aaggcggggt    360
tcacgcacgt gctgaacgcg gcccacggcc gctggaacgt ggacactggg cccgactact    420
accgcgacat ggacatccag taccacggcg tggaggccga cgacctgccc accttcgacc    480
tcagtgtctt cttctacccg gcggcagcct tcatcgacag agcgctaagc gacgaccaca    540
gtaagatcct ggttcactgc gtcatgggcc gcagccggtc agccaccctg gtcctggcct    600
acctgatgat ccacaaggac atgaccctgg tggacgccat ccagcaagtg gccaagaacc    660
gctgcgtcct cccgaaccgg ggctttttga agcagctccg ggagctggac aagcagctgg    720
tgcagcagag gcgacggtcc cagcgccagg acggtgagga ggaggatggc agggagctgt    780
aggcccgact cacagggcca gcagaggcac ttggggacag aggggagagg cagaacatag    840
ccctggccta ggactccaga gaagggatgg tgaaaccgaa gctcgactct tccaaaccat    900
cttgttcaac ttccccatgt gtgctgggga cagggaggac ccagagctgc ccccgggcag    960
agctgagcgc tcagcctctc agcaaaatgg gagggacggg ctccccggct ctgggtcaca   1020
gaggagcatg ccacgctgca ccaagtctcc tgctttggtt ttgttttttt ggtgagaagg   1080
aagagggaaa aagattttta aaatgtgtag gcagtatgtt gtgattaaac gtttggcttt   1140
gtccaaaaaa aaaaaaaaaa aaaaa                                         1165
```

<210> SEQ ID NO 787
<211> LENGTH: 220
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 787

```
Met Thr Ser Gly Glu Val Lys Thr Ser Leu Lys Asn Ala Tyr Ser Ser
1               5                   10                  15

Ala Lys Arg Leu Ser Pro Lys Met Glu Glu Glu Gly Glu Glu Glu Asp
            20                  25                  30

Tyr Cys Thr Pro Gly Ala Phe Glu Leu Glu Arg Leu Phe Trp Lys Gly
        35                  40                  45

Ser Pro Gln Tyr Thr His Val Asn Glu Val Trp Pro Lys Leu Tyr Ile
    50                  55                  60

Gly Asp Glu Ala Thr Ala Leu Asp Arg Tyr Arg Leu Gln Lys Ala Gly
65                  70                  75                  80

Phe Thr His Val Leu Asn Ala Ala His Gly Arg Trp Asn Val Asp Thr
                85                  90                  95

Gly Pro Asp Tyr Tyr Arg Asp Met Asp Ile Gln Tyr His Gly Val Glu
```

-continued

```
            100                 105                 110

Ala Asp Asp Leu Pro Thr Phe Asp Leu Ser Val Phe Phe Tyr Pro Ala
        115                 120                 125

Ala Ala Phe Ile Asp Arg Ala Leu Ser Asp Asp His Ser Lys Ile Leu
    130                 135                 140

Val His Cys Val Met Gly Arg Ser Arg Ser Ala Thr Leu Val Leu Ala
145                 150                 155                 160

Tyr Leu Met Ile His Lys Asp Met Thr Leu Val Asp Ala Ile Gln Gln
                165                 170                 175

Val Ala Lys Asn Arg Cys Val Leu Pro Asn Arg Gly Phe Leu Lys Gln
            180                 185                 190

Leu Arg Glu Leu Asp Lys Gln Leu Val Gln Gln Arg Arg Arg Ser Gln
        195                 200                 205

Arg Gln Asp Gly Glu Glu Glu Asp Gly Arg Glu Leu
    210                 215                 220
```

<210> SEQ ID NO 788
<211> LENGTH: 2276
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 788

```
ctgccccgcg tccggtcccg agcgggcctc cctcgggcca gcccgatgtg accgagccca     60

gcggagcctg agcaaggagc gggtccgtcg cggagccgga gggcgggagg aacatgacat    120

cgcggagatg gtttcaccca aatatcactg gtgtggaggc agaaaaccta ctgttgacaa    180

gaggagttga tggcagtttt ttggcaaggc ctagtaaaag taaccctgga gacttcacac    240

tttccgttag aagaaatgga gctgtcaccc acatcaagat tcagaacact ggtgattact    300

atgacctgta tggaggggag aaatttgcca ctttggctga gttggtccag tattacatgg    360

aacatcacgg gcaattaaaa gagaagaatg gagatgtcat tgagcttaaa tatcctctga    420

actgtgcaga tcctacctct gaaaggtggt ttcatggaca tctctctggg aaagaagcag    480

agaaattatt aactgaaaaa ggaaaacatg gtagttttct tgtacgagag agccagagcc    540

accctggaga ttttgttctt tctgtgcgca ctggtgatga caaaggggag agcaatgacg    600

gcaagtctaa agtgacccat gttatgattc gctgtcagga actgaaatac gacgttggtg    660

gaggagaacg gtttgattct ttgacagatc ttgtggaaca ttataagaag aatcctatgg    720

tggaaacatt gggtacagta ctacaactca agcagcccct taacacgact cgtataaatg    780

ctgctgaaat agaaagcaga gttcgagaac taagcaaatt agctgagacc acagataaag    840

tcaaacaagg cttttgggaa gaatttgaga cactacaaca acaggagtgc aaacttctct    900

acagccgaaa agagggtcaa aggcaagaaa acaaaaacaa aaatagatat aaaaacatcc    960

tgccctttga tcataccagg gttgtcctac acgatggtga tcccaatgag cctgtttcag   1020

attacatcaa tgcaaatatc atcatgcctg aatttgaaac caagtgcaac aattcaaagc   1080

ccaaaaagag ttacattgcc acacaaggct gcctgcaaaa cacggtgaat gacttttggc   1140

ggatggtgtt ccaagaaaac tcccgagtga ttgtcatgac aacgaaagaa gtggagagag   1200

gaaagagtaa atgtgtcaaa tactggcctg atgagtatgc tctaaaagaa tatggcgtca   1260

tgcgtgttag gaacgtcaaa gaaagcgccg ctcatgacta tacgctaaga gaacttaaac   1320

tttcaaaggt tggacaaggg aatacggaga gaacggtctg gcaataccac tttcggacct   1380
```

-continued

ggccggacca cggcgtgccc agcgaccctg ggggcgtgct ggacttcctg gaggaggtgc 1440

accataagca ggagagcatc atggatgcag ggccggtcgt ggtgcactgc agtgctggaa 1500

ttggccggac agggacgttc attgtgattg atattcttat tgacatcatc agagagaaag 1560

gtgttgactg cgatattgac gttcccaaaa ccatccagat ggtgcggtct cagaggtcag 1620

ggatggtcca gacagaagca cagtaccgat ttatctatat ggcggtccag cattatattg 1680

aaacactaca gcgcaggatt gaagaagagc agaaaagcaa gaggaaaggg cacgaatata 1740

caaatattaa gtattctcta gcggaccaga cgagtggaga tcagagccct ctcccgcctt 1800

gtactccaac gccaccctgt gcagaaatga gagaagacag tgctagagtc tatgaaaacg 1860

tgggcctgat gcaacagcag aaaagtttca gatgagaaaa cctgccaaaa cttcagcaca 1920

gaaatagatg tggactttca ccctctccct aaaaagatca agaacagacg caagaaagtt 1980

tatgtgaaga cagaatttgg atttggaagg cttgcaatgt ggttgactac cttttgataa 2040

gcaaaatttg aaaccattta aagaccactg tattttaact caacaatacc tgcttcccaa 2100

ttactcattt cctcagataa gaagaaatca tctctacaat gtagacaaca ttatatttta 2160

tagaatttgt ttgaaattga ggaagcagtt aaattgtgcg ctgtattttg cagattatgg 2220

ggattcaaat tctagtaata ggcttttta tttttatttt tatacccta accagg 2276

<210> SEQ ID NO 789
<211> LENGTH: 593
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 789

Met Thr Ser Arg Arg Trp Phe His Pro Asn Ile Thr Gly Val Glu Ala
1   5   10   15

Glu Asn Leu Leu Leu Thr Arg Gly Val Asp Gly Ser Phe Leu Ala Arg
            20   25   30

Pro Ser Lys Ser Asn Pro Gly Asp Phe Thr Leu Ser Val Arg Arg Asn
        35   40   45

Gly Ala Val Thr His Ile Lys Ile Gln Asn Thr Gly Asp Tyr Tyr Asp
    50   55   60

Leu Tyr Gly Gly Glu Lys Phe Ala Thr Leu Ala Glu Leu Val Gln Tyr
65   70   75   80

Tyr Met Glu His His Gly Gln Leu Lys Glu Lys Asn Gly Asp Val Ile
                85   90   95

Glu Leu Lys Tyr Pro Leu Asn Cys Ala Asp Pro Thr Ser Glu Arg Trp
            100   105   110

Phe His Gly His Leu Ser Gly Lys Glu Ala Glu Lys Leu Leu Thr Glu
        115   120   125

Lys Gly Lys His Gly Ser Phe Leu Val Arg Glu Ser Gln Ser His Pro
    130   135   140

Gly Asp Phe Val Leu Ser Val Arg Thr Gly Asp Asp Lys Gly Glu Ser
145   150   155   160

Asn Asp Gly Lys Ser Lys Val Thr His Val Met Ile Arg Cys Gln Glu
                165   170   175

Leu Lys Tyr Asp Val Gly Gly Gly Glu Arg Phe Asp Ser Leu Thr Asp
            180   185   190

Leu Val Glu His Tyr Lys Lys Asn Pro Met Val Glu Thr Leu Gly Thr

-continued

```
        195                 200                 205

Val Leu Gln Leu Lys Gln Pro Leu Asn Thr Thr Arg Ile Asn Ala Ala
    210                 215                 220

Glu Ile Glu Ser Arg Val Arg Glu Leu Ser Lys Leu Ala Glu Thr Thr
225                 230                 235                 240

Asp Lys Val Lys Gln Gly Phe Trp Glu Glu Phe Glu Thr Leu Gln Gln
                245                 250                 255

Gln Glu Cys Lys Leu Leu Tyr Ser Arg Lys Glu Gly Gln Arg Gln Glu
            260                 265                 270

Asn Lys Asn Lys Asn Arg Tyr Lys Asn Ile Leu Pro Phe Asp His Thr
        275                 280                 285

Arg Val Val Leu His Asp Gly Asp Pro Asn Glu Pro Val Ser Asp Tyr
    290                 295                 300

Ile Asn Ala Asn Ile Ile Met Pro Glu Phe Glu Thr Lys Cys Asn Asn
305                 310                 315                 320

Ser Lys Pro Lys Lys Ser Tyr Ile Ala Thr Gln Gly Cys Leu Gln Asn
                325                 330                 335

Thr Val Asn Asp Phe Trp Arg Met Val Phe Gln Glu Asn Ser Arg Val
            340                 345                 350

Ile Val Met Thr Thr Lys Glu Val Glu Arg Gly Lys Ser Lys Cys Val
        355                 360                 365

Lys Tyr Trp Pro Asp Glu Tyr Ala Leu Lys Glu Tyr Gly Val Met Arg
    370                 375                 380

Val Arg Asn Val Lys Glu Ser Ala Ala His Asp Tyr Thr Leu Arg Glu
385                 390                 395                 400

Leu Lys Leu Ser Lys Val Gly Gln Gly Asn Thr Glu Arg Thr Val Trp
                405                 410                 415

Gln Tyr His Phe Arg Thr Trp Pro Asp His Gly Val Pro Ser Asp Pro
            420                 425                 430

Gly Gly Val Leu Asp Phe Leu Glu Glu Val His His Lys Gln Glu Ser
        435                 440                 445

Ile Met Asp Ala Gly Pro Val Val Val His Cys Ser Ala Gly Ile Gly
    450                 455                 460

Arg Thr Gly Thr Phe Ile Val Ile Asp Ile Leu Ile Asp Ile Ile Arg
465                 470                 475                 480

Glu Lys Gly Val Asp Cys Asp Ile Asp Val Pro Lys Thr Ile Gln Met
                485                 490                 495

Val Arg Ser Gln Arg Ser Gly Met Val Gln Thr Glu Ala Gln Tyr Arg
            500                 505                 510

Phe Ile Tyr Met Ala Val Gln His Tyr Ile Glu Thr Leu Gln Arg Arg
        515                 520                 525

Ile Glu Glu Glu Gln Lys Ser Lys Arg Lys Gly His Glu Tyr Thr Asn
    530                 535                 540

Ile Lys Tyr Ser Leu Ala Asp Gln Thr Ser Gly Asp Gln Ser Pro Leu
545                 550                 555                 560

Pro Pro Cys Thr Pro Thr Pro Pro Cys Ala Glu Met Arg Glu Asp Ser
                565                 570                 575

Ala Arg Val Tyr Glu Asn Val Gly Leu Met Gln Gln Gln Lys Ser Phe
            580                 585                 590

Arg
```

-continued

<210> SEQ ID NO 790
<211> LENGTH: 2121
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 790

cgccaggcct ggagggggt ctgtgcgcgg ccggctggct ctgccccgcg tccggtcccg 60

agcgggcctc cctcgggcca gcccgatgtg accgagccca gcggagcctg agcaaggagc 120

gggtccgtcg cggagccgga gggcgggagg aacatgacat cgcggagatg gtttcaccca 180

aatatcactg gtgtggaggc agaaaaccta ctgttgacaa gaggagttga tggcagtttt 240

ttggcaaggc ctagtaaaag taaccctgga gacttcacac tttccgttag aagaaatgga 300

gctgtcaccc acatcaagat tcagaacact ggtgattact atgacctgta tggaggggag 360

aaatttgcca ctttggctga gttggtccag tattacatgg aacatcacgg gcaattaaaa 420

gagaagaatg gagatgtcat tgagcttaaa tatcctctga actgtgcaga tcctacctct 480

gaaaggtggt ttcatggaca tctctctggg aaagaagcag agaaattatt aactgaaaaa 540

ggaaaacatg gtagttttct tgtacgagag agccagagcc accctggaga ttttgttctt 600

tctgtgcgca ctggtgatga caaaggggag agcaatgacg gcaagtctaa agtgacccat 660

gttatgattc gctgtcagga actgaaatac gacgttggtg gaggagaacg gtttgattct 720

ttgacagatc ttgtggaaca ttataagaag aatcctatgg tggaaacatt gggtacagta 780

ctacaactca agcagcccct taacacgact cgtataaatg ctgctgaaat agaaagcaga 840

gttcgagaac taagcaaatt agctgagacc acagataaag tcaaacaagg cttttgggaa 900

gaatttgaga cactacaaca acaggagtgc aaacttctct acagccgaaa agagggtcaa 960

aggcaagaaa acaaaaacaa aaatagatat aaaaacatcc tgccctttga tcataccagg 1020

gttgtcctac acgatggtga tcccaatgag cctgtttcag attacatcaa tgcaaatatc 1080

atcatgcctg aatttgaaac caagtgcaac aattcaaagc ccaaaaagag ttacattgcc 1140

acacaaggct gcctgcaaaa cacggtgaat gacttttggc ggatggtgtt ccaagaaaac 1200

tcccgagtga ttgtcatgac aacgaaagaa gtggagagag gaaagagtaa atgtgtcaaa 1260

tactggcctg atgagtatgc tctaaaagaa tatggcgtca tgcgtgttag gaacgtcaaa 1320

gaaagcgccg ctcatgacta tacgctaaga gaacttaaac tttcaaaggt tggacaaggg 1380

aatacggaga gaacggtctg gcaataccac tttcggacct ggccggacca cggcgtgccc 1440

agcgaccctg gggcgtgct ggacttcctg gaggaggtgc accataagca ggagagcatc 1500

atggatgcag ggccggtcgt ggtgcactgc agtgctggaa ttggccggac agggacgttc 1560

attgtgattg atattcttat tgacatcatc agagagaaag gtgttgactg cgatattgac 1620

gttcccaaaa ccatccagat ggtgcggtct cagaggtcag ggatggtcca gacagaagca 1680

cagtaccgat ttatctatat ggcggtccag cattatattg aaacactaca gcgcaggatt 1740

gaagaagagc agaaaagcaa gaggaaaggg cacgaatata caaatattaa gtattctcta 1800

gcggaccaga cgagtggaga tcagagccct ctcccgcctt gtactccaac gccaccctgt 1860

gcagaaatga gagaagacag tgctagagtc tatgaaaacg tgggcctgat gcaacagcag 1920

aaaagtttca gatgagaaaa cctgccaaaa cttcagcaca gaaatagatg tggactttca 1980

ccctctccct aaaaagatca agaacagacg caagaaagtt tatgtgaaga cagaatttgg 2040

-continued

```
atttggaagg cttgcaatgt ggttgactac cttttgataa gcaaaatttg aaaccattta   2100

aagaccactg tattttaact c                                              2121


<210> SEQ ID NO 791
<211> LENGTH: 593
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 791

Met Thr Ser Arg Arg Trp Phe His Pro Asn Ile Thr Gly Val Glu Ala
1               5                   10                  15

Glu Asn Leu Leu Leu Thr Arg Gly Val Asp Gly Ser Phe Leu Ala Arg
            20                  25                  30

Pro Ser Lys Ser Asn Pro Gly Asp Phe Thr Leu Ser Val Arg Arg Asn
        35                  40                  45

Gly Ala Val Thr His Ile Lys Ile Gln Asn Thr Gly Asp Tyr Tyr Asp
    50                  55                  60

Leu Tyr Gly Gly Glu Lys Phe Ala Thr Leu Ala Glu Leu Val Gln Tyr
65                  70                  75                  80

Tyr Met Glu His His Gly Gln Leu Lys Glu Lys Asn Gly Asp Val Ile
                85                  90                  95

Glu Leu Lys Tyr Pro Leu Asn Cys Ala Asp Pro Thr Ser Glu Arg Trp
            100                 105                 110

Phe His Gly His Leu Ser Gly Lys Glu Ala Glu Lys Leu Leu Thr Glu
        115                 120                 125

Lys Gly Lys His Gly Ser Phe Leu Val Arg Glu Ser Gln Ser His Pro
    130                 135                 140

Gly Asp Phe Val Leu Ser Val Arg Thr Gly Asp Asp Lys Gly Glu Ser
145                 150                 155                 160

Asn Asp Gly Lys Ser Lys Val Thr His Val Met Ile Arg Cys Gln Glu
                165                 170                 175

Leu Lys Tyr Asp Val Gly Gly Gly Glu Arg Phe Asp Ser Leu Thr Asp
            180                 185                 190

Leu Val Glu His Tyr Lys Lys Asn Pro Met Val Glu Thr Leu Gly Thr
        195                 200                 205

Val Leu Gln Leu Lys Gln Pro Leu Asn Thr Thr Arg Ile Asn Ala Ala
    210                 215                 220

Glu Ile Glu Ser Arg Val Arg Glu Leu Ser Lys Leu Ala Glu Thr Thr
225                 230                 235                 240

Asp Lys Val Lys Gln Gly Phe Trp Glu Glu Phe Glu Thr Leu Gln Gln
                245                 250                 255

Gln Glu Cys Lys Leu Leu Tyr Ser Arg Lys Glu Gly Gln Arg Gln Glu
            260                 265                 270

Asn Lys Asn Lys Asn Arg Tyr Lys Asn Ile Leu Pro Phe Asp His Thr
        275                 280                 285

Arg Val Val Leu His Asp Gly Asp Pro Asn Glu Pro Val Ser Asp Tyr
    290                 295                 300

Ile Asn Ala Asn Ile Ile Met Pro Glu Phe Glu Thr Lys Cys Asn Asn
305                 310                 315                 320

Ser Lys Pro Lys Lys Ser Tyr Ile Ala Thr Gln Gly Cys Leu Gln Asn
                325                 330                 335

Thr Val Asn Asp Phe Trp Arg Met Val Phe Gln Glu Asn Ser Arg Val
```

-continued

```
            340                 345                 350

Ile Val Met Thr Thr Lys Glu Val Glu Arg Gly Lys Ser Lys Cys Val
        355                 360                 365

Lys Tyr Trp Pro Asp Glu Tyr Ala Leu Lys Glu Tyr Gly Val Met Arg
    370                 375                 380

Val Arg Asn Val Lys Glu Ser Ala Ala His Asp Tyr Thr Leu Arg Glu
385                 390                 395                 400

Leu Lys Leu Ser Lys Val Gly Gln Gly Asn Thr Glu Arg Thr Val Trp
                405                 410                 415

Gln Tyr His Phe Arg Thr Trp Pro Asp His Gly Val Pro Ser Asp Pro
            420                 425                 430

Gly Gly Val Leu Asp Phe Leu Glu Glu Val His His Lys Gln Glu Ser
        435                 440                 445

Ile Met Asp Ala Gly Pro Val Val Val His Cys Ser Ala Gly Ile Gly
    450                 455                 460

Arg Thr Gly Thr Phe Ile Val Ile Asp Ile Leu Ile Asp Ile Ile Arg
465                 470                 475                 480

Glu Lys Gly Val Asp Cys Asp Ile Asp Val Pro Lys Thr Ile Gln Met
                485                 490                 495

Val Arg Ser Gln Arg Ser Gly Met Val Gln Thr Glu Ala Gln Tyr Arg
            500                 505                 510

Phe Ile Tyr Met Ala Val Gln His Tyr Ile Glu Thr Leu Gln Arg Arg
        515                 520                 525

Ile Glu Glu Glu Gln Lys Ser Lys Arg Lys Gly His Glu Tyr Thr Asn
    530                 535                 540

Ile Lys Tyr Ser Leu Ala Asp Gln Thr Ser Gly Asp Gln Ser Pro Leu
545                 550                 555                 560

Pro Pro Cys Thr Pro Thr Pro Pro Cys Ala Glu Met Arg Glu Asp Ser
                565                 570                 575

Ala Arg Val Tyr Glu Asn Val Gly Leu Met Gln Gln Gln Lys Ser Phe
            580                 585                 590

Arg
```

<210> SEQ ID NO 792
<211> LENGTH: 2654
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 792

```
agccggagct ggagccgagg cggcggcggg acgcggccgg ccggacaaat ttcctgctag      60

gctgcggacg agcgggcggc aggagccggc gcgagcggct tcaggaaccc acggcctctg     120

cgcgtccccg cgacccttct tcgcgcccgg cgaagacagc cgggcgcccc ggagggcggc     180

gggcaggcgc ccgggagatg cggagcctcc gctgcagcgc gatctgcgcg accagaccgg     240

ccccccccgag actatagcct tcactttccc tcggtccacc atggagccct tgtgtccact     300

cctgctggtg ggttttagct tgccgctcgc cagggctctc aggggcaacg agaccactgc     360

cgacagcaac gagacaacca cgacctcagg ccctccggac ccgggcgcct cccagccgct     420

gctggcctgg ctgctactgc cgctgctgct cctcctcctc gtgctccttc tcgccgccta     480

cttcttcagg ttcaggaagc agaggaaagc tgtggtcagc accagcgaca agaagatgcc     540

caacggaatc ttggaggagc aagagcagca aagggtgatg ctgctcagca ggtcaccctc     600
```

-continued

```
agggcccaag aagtattttc ccatccccgt ggagcacctg gaggaggaga tccgtatcag    660
atccgccgac gactgcaagc agtttcggga ggagttcaac tcattgccat ctggacacat    720
acaaggaact tttgaactgg caaataaaga agaaaacaga gaaaaaaaca gatatcccaa    780
catccttccc aatgaccatt ctagggtgat tctgagccaa ctggatggaa ttccctgttc    840
agactacatc aatgcttcct acatagatgg ttacaaagag aagaataaat tcatagcagc    900
tcaaggtccc aaacaggaaa cggttaacga cttctggaga atggtctggg agcaaaagtc    960
tgcgaccatc gtcatgttaa caaacttgaa agaaaggaaa gaggaaaagt gccatcagta   1020
ctggcccgac caaggctgct ggacctatgg aaacatccgg gtgtgcgtgg aggactgcgt   1080
ggttttggtc gactacacca tccggaagtt ctgcatacag ccacagctcc ccgacggctg   1140
caaagccccc aggctggtct cacagctgca cttcaccagc tggcccgact tcggagtgcc   1200
ttttaccccc attgggatgc tgaagttcct caagaaagta aagacgctca accccgtgca   1260
cgctgggccc atcgtggtcc actgtagcgc gggcgtgggc cggacgggca ccttcattgt   1320
gatcgatgcc atgatggcca tgatgcacgc ggagcagaag gtggatgtgt ttgaatttgt   1380
gtctcgaatc cgtaatcagc gccctcagat ggttcaaacg gatatgcagt acacgttcat   1440
ctaccaagcc ttactcgagt actacctcta cggggacaca gagctggacg tgtcctccct   1500
ggagaagcac ctgcagacca tgcacggcac caccacccac ttcgacaaga tcgggctgga   1560
ggaggagttc aggaaattga caaatgtccg gatcatgaag gagaacatga ggacgggcaa   1620
cttgccggca aacatgaaga aggccagggt catccagatc atcccgtatg acttcaaccg   1680
agtgatcctt tccatgaaaa ggggtcaaga atacacagac tacatcaacg catccttcat   1740
agacggctac cgacagaagg actatttcat cgccacccag gggccactgg cacacacggt   1800
tgaggacttc tggaggatga tctgggaatg gaaatcccac actatcgtga tgctgacgga   1860
ggtgcaggag agagagcagg ataaatgcta ccagtattgg ccaaccgagg gctcagttac   1920
tcatggagaa ataacgattg agataaagaa tgataccctt tcagaagcca tcagtatacg   1980
agactttctg gtcactctca atcagcccca ggcccgccag gaggagcagg tccgagtagt   2040
gcgccagttt cacttccacg gctggcctga gatcgggatt cccgccgagg gcaaaggcat   2100
gattgacctc atcgcagccg tgcagaagca gcagcagcag acaggcaacc accccatcac   2160
cgtgcactgc agtgccggag ctgggcgaac aggtacattc atagccctca gcaacatttt   2220
ggagcgagta aaagccgagg gacttttaga tgtatttcaa gctgtgaaga gtttacgact   2280
tcagagacca catatggtgc aaaccctgga acagtatgaa ttctgctaca aagtggtaca   2340
agattttatt gatatatttt ctgattatgc taatttcaaa tgaagattcc tgccttaaaa   2400
tattttttaa tttaatggtc agtatatttt gtaaaaatca tgttaattta tttcatagtt   2460
gacattaata tcttccctaa tttctttgta tatattttgt tatgccttaa aggccacctg   2520
ctatacagtt gttaaatctt aaatatgctt tttaaaaatt ggaataatgt attaaggtca   2580
aataatatcc cataaaatat atatttctgc taatattagt aaatatctta atttttaaaa   2640
aaaaaaaaaa aaaa                                                     2654
```

<210> SEQ ID NO 793
<211> LENGTH: 700
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

-continued

<400> SEQUENCE: 793

```
Met Glu Pro Leu Cys Pro Leu Leu Leu Val Gly Phe Ser Leu Pro Leu
1               5                   10                  15

Ala Arg Ala Leu Arg Gly Asn Glu Thr Thr Ala Asp Ser Asn Glu Thr
            20                  25                  30

Thr Thr Thr Ser Gly Pro Pro Asp Pro Gly Ala Ser Gln Pro Leu Leu
        35                  40                  45

Ala Trp Leu Leu Leu Pro Leu Leu Leu Leu Leu Leu Val Leu Leu Leu
    50                  55                  60

Ala Ala Tyr Phe Phe Arg Phe Arg Lys Gln Arg Lys Ala Val Val Ser
65                  70                  75                  80

Thr Ser Asp Lys Lys Met Pro Asn Gly Ile Leu Glu Glu Gln Glu Gln
                85                  90                  95

Gln Arg Val Met Leu Leu Ser Arg Ser Pro Ser Gly Pro Lys Lys Tyr
            100                 105                 110

Phe Pro Ile Pro Val Glu His Leu Glu Glu Glu Ile Arg Ile Arg Ser
        115                 120                 125

Ala Asp Asp Cys Lys Gln Phe Arg Glu Glu Phe Asn Ser Leu Pro Ser
    130                 135                 140

Gly His Ile Gln Gly Thr Phe Glu Leu Ala Asn Lys Glu Glu Asn Arg
145                 150                 155                 160

Glu Lys Asn Arg Tyr Pro Asn Ile Leu Pro Asn Asp His Ser Arg Val
                165                 170                 175

Ile Leu Ser Gln Leu Asp Gly Ile Pro Cys Ser Asp Tyr Ile Asn Ala
            180                 185                 190

Ser Tyr Ile Asp Gly Tyr Lys Glu Lys Asn Lys Phe Ile Ala Ala Gln
        195                 200                 205

Gly Pro Lys Gln Glu Thr Val Asn Asp Phe Trp Arg Met Val Trp Glu
    210                 215                 220

Gln Lys Ser Ala Thr Ile Val Met Leu Thr Asn Leu Lys Glu Arg Lys
225                 230                 235                 240

Glu Glu Lys Cys His Gln Tyr Trp Pro Asp Gln Gly Cys Trp Thr Tyr
                245                 250                 255

Gly Asn Ile Arg Val Cys Val Glu Asp Cys Val Val Leu Val Asp Tyr
            260                 265                 270

Thr Ile Arg Lys Phe Cys Ile Gln Pro Gln Leu Pro Asp Gly Cys Lys
        275                 280                 285

Ala Pro Arg Leu Val Ser Gln Leu His Phe Thr Ser Trp Pro Asp Phe
    290                 295                 300

Gly Val Pro Phe Thr Pro Ile Gly Met Leu Lys Phe Leu Lys Lys Val
305                 310                 315                 320

Lys Thr Leu Asn Pro Val His Ala Gly Pro Ile Val Val His Cys Ser
                325                 330                 335

Ala Gly Val Gly Arg Thr Gly Thr Phe Ile Val Ile Asp Ala Met Met
            340                 345                 350

Ala Met Met His Ala Glu Gln Lys Val Asp Val Phe Glu Phe Val Ser
        355                 360                 365

Arg Ile Arg Asn Gln Arg Pro Gln Met Val Gln Thr Asp Met Gln Tyr
    370                 375                 380

Thr Phe Ile Tyr Gln Ala Leu Leu Glu Tyr Tyr Leu Tyr Gly Asp Thr
```

-continued

```
385                 390                 395                 400

Glu Leu Asp Val Ser Ser Leu Glu Lys His Leu Gln Thr Met His Gly
                405                 410                 415

Thr Thr Thr His Phe Asp Lys Ile Gly Leu Glu Glu Glu Phe Arg Lys
            420                 425                 430

Leu Thr Asn Val Arg Ile Met Lys Glu Asn Met Arg Thr Gly Asn Leu
        435                 440                 445

Pro Ala Asn Met Lys Lys Ala Arg Val Ile Gln Ile Ile Pro Tyr Asp
    450                 455                 460

Phe Asn Arg Val Ile Leu Ser Met Lys Arg Gly Gln Glu Tyr Thr Asp
465                 470                 475                 480

Tyr Ile Asn Ala Ser Phe Ile Asp Gly Tyr Arg Gln Lys Asp Tyr Phe
                485                 490                 495

Ile Ala Thr Gln Gly Pro Leu Ala His Thr Val Glu Asp Phe Trp Arg
            500                 505                 510

Met Ile Trp Glu Trp Lys Ser His Thr Ile Val Met Leu Thr Glu Val
        515                 520                 525

Gln Glu Arg Glu Gln Asp Lys Cys Tyr Gln Tyr Trp Pro Thr Glu Gly
    530                 535                 540

Ser Val Thr His Gly Glu Ile Thr Ile Glu Ile Lys Asn Asp Thr Leu
545                 550                 555                 560

Ser Glu Ala Ile Ser Ile Arg Asp Phe Leu Val Thr Leu Asn Gln Pro
                565                 570                 575

Gln Ala Arg Gln Glu Glu Gln Val Arg Val Val Arg Gln Phe His Phe
            580                 585                 590

His Gly Trp Pro Glu Ile Gly Ile Pro Ala Glu Gly Lys Gly Met Ile
        595                 600                 605

Asp Leu Ile Ala Ala Val Gln Lys Gln Gln Gln Gln Thr Gly Asn His
    610                 615                 620

Pro Ile Thr Val His Cys Ser Ala Gly Ala Gly Arg Thr Gly Thr Phe
625                 630                 635                 640

Ile Ala Leu Ser Asn Ile Leu Glu Arg Val Lys Ala Glu Gly Leu Leu
                645                 650                 655

Asp Val Phe Gln Ala Val Lys Ser Leu Arg Leu Gln Arg Pro His Met
            660                 665                 670

Val Gln Thr Leu Glu Gln Tyr Glu Phe Cys Tyr Lys Val Val Gln Asp
        675                 680                 685

Phe Ile Asp Ile Phe Ser Asp Tyr Ala Asn Phe Lys
    690                 695                 700

<210> SEQ ID NO 794
<211> LENGTH: 2263
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 794

ctgagaggct gggtggctgg gcctgggaga cacacagagg ccaggcctta gcgcggctca      60

gccatgagca acaggagtag cttttcccgg ctcacctggt tcaggaagca gaggaaagct     120

gtggtcagca ccagcgacaa gaagatgccc aacggaatct tggaggagca agagcagcaa     180

agggtgatgc tgctcagcag gtcaccctca gggcccaaga agtattttcc catccccgtg     240

gagcacctgg aggaggagat ccgtatcaga tccgccgacg actgcaagca gtttcgggag     300
```

-continued

```
gagttcaact cattgccatc tggacacata caaggaactt ttgaactggc aaataaagaa    360
gaaaacagag aaaaaaacag atatcccaac atccttccca atgaccattc tagggtgatt    420
ctgagccaac tggatggaat tccctgttca gactacatca atgcttccta catagatggt    480
tacaaagaga agaataaatt catagcagct caaggtccca aacaggaaac ggttaacgac    540
ttctggagaa tggtctggga gcaaaagtct gcgaccatcg tcatgttaac aaacttgaaa    600
gaaaggaaag aggaaaagtg ccatcagtac tggcccgacc aaggctgctg gacctatgga    660
aacatccggg tgtgcgtgga ggactgcgtg gttttggtcg actacaccat ccggaagttc    720
tgcatacagc cacagctccc cgacggctgc aaagccccca ggctggtctc acagctgcac    780
ttcaccagct ggcccgactt cggagtgcct tttacccca ttgggatgct gaagttcctc    840
aagaaagtaa agacgctcaa ccccgtgcac gctgggccca tcgtggtcca ctgtagcgcg    900
ggcgtgggcc ggacgggcac cttcattgtg atcgatgcca tgatggccat gatgcacgcg    960
gagcagaagg tggatgtgtt tgaatttgtg tctcgaatcc gtaatcagcg ccctcagatg   1020
gttcaaacgg atatgcagta cacgttcatc taccaagcct tactcgagta ctacctctac   1080
ggggacacag agctggacgt gtcctccctg gagaagcacc tgcagaccat gcacggcacc   1140
accacccact tcgacaagat cgggctggag gaggagttca ggaaattgac aaatgtccgg   1200
atcatgaagg agaacatgag gacgggcaac ttgccggcaa acatgaagaa ggccagggtc   1260
atccagatca tcccgtatga cttcaaccga gtgatccttt ccatgaaaag ggtcaagaa   1320
tacacagact acatcaacgc atccttcata gacggctacc gacagaagga ctatttcatc   1380
gccacccagg ggccactggc acacacggtt gaggacttct ggaggatgat ctgggaatgg   1440
aaatcccaca ctatcgtgat gctgacggag gtgcaggaga gagagcagga taaatgctac   1500
cagtattggc caaccgaggg ctcagttact catggagaaa taacgattga gataaagaat   1560
gatacccttt cagaagccat cagtatacga gactttctgg tcactctcaa tcagccccag   1620
gcccgccagg aggagcaggt ccgagtagtg cgccagtttc acttccacgg ctggcctgag   1680
atcgggattc ccgccgaggg caaaggcatg attgacctca tcgcagccgt gcagaagcag   1740
cagcagcaga caggcaacca ccccatcacc gtgcactgca gtgccggagc tgggcgaaca   1800
ggtacattca tagccctcag caacattttg gagcgagtaa aagccgaggg acttttagat   1860
gtatttcaag ctgtgaagag tttacgactt cagagaccac atatggtgca aaccctggaa   1920
cagtatgaat tctgctacaa agtggtacaa gattttattg atatattttc tgattatgct   1980
aatttcaaat gaagattcct gccttaaaat atttttttaat ttaatggtca gtatattttg   2040
taaaaatcat gttaatttat ttcatagttg acattaatat cttccctaat ttctttgtat   2100
atattttgtt atgccttaaa ggccacctgc tatacagttg ttaaatctta aatatgcttt   2160
ttaaaaattg gaataatgta ttaaggtcaa ataatatccc ataaaatata tatttctgct   2220
aatattagta aatatcttaa tttttaaaaa aaaaaaaaaa aaa                    2263
```

<210> SEQ ID NO 795
<211> LENGTH: 642
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 795

Met Ser Asn Arg Ser Ser Phe Ser Arg Leu Thr Trp Phe Arg Lys Gln

-continued

```
1               5                   10                  15

Arg Lys Ala Val Val Ser Thr Ser Asp Lys Lys Met Pro Asn Gly Ile
                20                  25                  30

Leu Glu Glu Gln Glu Gln Gln Arg Val Met Leu Leu Ser Arg Ser Pro
        35                  40                  45

Ser Gly Pro Lys Lys Tyr Phe Pro Ile Pro Val Glu His Leu Glu Glu
    50                  55                  60

Glu Ile Arg Ile Arg Ser Ala Asp Asp Cys Lys Gln Phe Arg Glu Glu
65                  70                  75                  80

Phe Asn Ser Leu Pro Ser Gly His Ile Gln Gly Thr Phe Glu Leu Ala
                85                  90                  95

Asn Lys Glu Glu Asn Arg Glu Lys Asn Arg Tyr Pro Asn Ile Leu Pro
            100                 105                 110

Asn Asp His Ser Arg Val Ile Leu Ser Gln Leu Asp Gly Ile Pro Cys
        115                 120                 125

Ser Asp Tyr Ile Asn Ala Ser Tyr Ile Asp Gly Tyr Lys Glu Lys Asn
    130                 135                 140

Lys Phe Ile Ala Ala Gln Gly Pro Lys Gln Glu Thr Val Asn Asp Phe
145                 150                 155                 160

Trp Arg Met Val Trp Glu Gln Lys Ser Ala Thr Ile Val Met Leu Thr
                165                 170                 175

Asn Leu Lys Glu Arg Lys Glu Glu Lys Cys His Gln Tyr Trp Pro Asp
            180                 185                 190

Gln Gly Cys Trp Thr Tyr Gly Asn Ile Arg Val Cys Val Glu Asp Cys
        195                 200                 205

Val Val Leu Val Asp Tyr Thr Ile Arg Lys Phe Cys Ile Gln Pro Gln
    210                 215                 220

Leu Pro Asp Gly Cys Lys Ala Pro Arg Leu Val Ser Gln Leu His Phe
225                 230                 235                 240

Thr Ser Trp Pro Asp Phe Gly Val Pro Phe Thr Pro Ile Gly Met Leu
                245                 250                 255

Lys Phe Leu Lys Lys Val Lys Thr Leu Asn Pro Val His Ala Gly Pro
            260                 265                 270

Ile Val Val His Cys Ser Ala Gly Val Gly Arg Thr Gly Thr Phe Ile
        275                 280                 285

Val Ile Asp Ala Met Met Ala Met Met His Ala Glu Gln Lys Val Asp
    290                 295                 300

Val Phe Glu Phe Val Ser Arg Ile Arg Asn Gln Arg Pro Gln Met Val
305                 310                 315                 320

Gln Thr Asp Met Gln Tyr Thr Phe Ile Tyr Gln Ala Leu Leu Glu Tyr
                325                 330                 335

Tyr Leu Tyr Gly Asp Thr Glu Leu Asp Val Ser Ser Leu Glu Lys His
            340                 345                 350

Leu Gln Thr Met His Gly Thr Thr Thr His Phe Asp Lys Ile Gly Leu
        355                 360                 365

Glu Glu Glu Phe Arg Lys Leu Thr Asn Val Arg Ile Met Lys Glu Asn
    370                 375                 380

Met Arg Thr Gly Asn Leu Pro Ala Asn Met Lys Lys Ala Arg Val Ile
385                 390                 395                 400

Gln Ile Ile Pro Tyr Asp Phe Asn Arg Val Ile Leu Ser Met Lys Arg
                405                 410                 415
```

-continued

Gly Gln Glu Tyr Thr Asp Tyr Ile Asn Ala Ser Phe Ile Asp Gly Tyr
420 425 430

Arg Gln Lys Asp Tyr Phe Ile Ala Thr Gln Gly Pro Leu Ala His Thr
435 440 445

Val Glu Asp Phe Trp Arg Met Ile Trp Glu Trp Lys Ser His Thr Ile
450 455 460

Val Met Leu Thr Glu Val Gln Glu Arg Glu Gln Asp Lys Cys Tyr Gln
465 470 475 480

Tyr Trp Pro Thr Glu Gly Ser Val Thr His Gly Glu Ile Thr Ile Glu
485 490 495

Ile Lys Asn Asp Thr Leu Ser Glu Ala Ile Ser Ile Arg Asp Phe Leu
500 505 510

Val Thr Leu Asn Gln Pro Gln Ala Arg Gln Glu Glu Gln Val Arg Val
515 520 525

Val Arg Gln Phe His Phe His Gly Trp Pro Glu Ile Gly Ile Pro Ala
530 535 540

Glu Gly Lys Gly Met Ile Asp Leu Ile Ala Ala Val Gln Lys Gln Gln
545 550 555 560

Gln Gln Thr Gly Asn His Pro Ile Thr Val His Cys Ser Ala Gly Ala
565 570 575

Gly Arg Thr Gly Thr Phe Ile Ala Leu Ser Asn Ile Leu Glu Arg Val
580 585 590

Lys Ala Glu Gly Leu Leu Asp Val Phe Gln Ala Val Lys Ser Leu Arg
595 600 605

Leu Gln Arg Pro His Met Val Gln Thr Leu Glu Gln Tyr Glu Phe Cys
610 615 620

Tyr Lys Val Val Gln Asp Phe Ile Asp Ile Phe Ser Asp Tyr Ala Asn
625 630 635 640

Phe Lys

<210> SEQ ID NO 796
<211> LENGTH: 844
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 796

gcacgagctg cagagggagg cggcactggt ctcgacgtgg ggcggccagc gatgaagccg 60

cccagttcaa tacaaacaag tgagtttgac tcatcagatg aagagcctat tgaagatgaa 120

cagactccaa ttcatatatc atggctatct ttgtcacgag tgaattgttc tcagtttctc 180

ggtttatgtg ctcttccagg ttgtaaattt aaagatgtta gaagaaatgt ccaaaaagat 240

acagaagaac taaagagctg tggtatacaa gacatatttg ttttctgcac cagaggggaa 300

ctgtcaaaat atagagtccc aaaccttctg gatctctacc agcaatgtgg aattatcacc 360

catcatcatc caatcgcaga tggagggact cctgacatag ccagctgctg tgaaataatg 420

gaagagctta caacctgcct taaaaattac cgaaaaacct taatacactg ctatggagga 480

cttgggagat cttgtcttgt agctgcttgt ctcctactat acctgtctga cacaatatca 540

ccagagcaag ccatagacag cctgcgagac ctaagaggat ccggggcaat acagaccatc 600

aagcaataca attatcttca tgagtttcgg gacaaattag ctgcacatct atcatcaaga 660

gattcacaat caagatctgt atcaagataa aggaattcaa atagcatata tatgaccatg 720

-continued

tctgaaatgt cagttctcta gcataatttg tattgaaatg aaaccaccag tgttatcaac 780

ttgaatgtaa atgtacatgt gcagatattc ctaaagtttt attgacaaaa aaaaaaaaaa 840

aaaa 844

<210> SEQ ID NO 797
<211> LENGTH: 212
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 797

Met Lys Pro Pro Ser Ser Ile Gln Thr Ser Glu Phe Asp Ser Ser Asp
1 5 10 15

Glu Glu Pro Ile Glu Asp Glu Gln Thr Pro Ile His Ile Ser Trp Leu
20 25 30

Ser Leu Ser Arg Val Asn Cys Ser Gln Phe Leu Gly Leu Cys Ala Leu
35 40 45

Pro Gly Cys Lys Phe Lys Asp Val Arg Arg Asn Val Gln Lys Asp Thr
50 55 60

Glu Glu Leu Lys Ser Cys Gly Ile Gln Asp Ile Phe Val Phe Cys Thr
65 70 75 80

Arg Gly Glu Leu Ser Lys Tyr Arg Val Pro Asn Leu Leu Asp Leu Tyr
85 90 95

Gln Gln Cys Gly Ile Ile Thr His His His Pro Ile Ala Asp Gly Gly
100 105 110

Thr Pro Asp Ile Ala Ser Cys Cys Glu Ile Met Glu Glu Leu Thr Thr
115 120 125

Cys Leu Lys Asn Tyr Arg Lys Thr Leu Ile His Cys Tyr Gly Gly Leu
130 135 140

Gly Arg Ser Cys Leu Val Ala Ala Cys Leu Leu Leu Tyr Leu Ser Asp
145 150 155 160

Thr Ile Ser Pro Glu Gln Ala Ile Asp Ser Leu Arg Asp Leu Arg Gly
165 170 175

Ser Gly Ala Ile Gln Thr Ile Lys Gln Tyr Asn Tyr Leu His Glu Phe
180 185 190

Arg Asp Lys Leu Ala Ala His Leu Ser Ser Arg Asp Ser Gln Ser Arg
195 200 205

Ser Val Ser Arg
210

<210> SEQ ID NO 798
<211> LENGTH: 1396
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 798

tgactatcca gctctgagag acgggagttt ggagttgccc gctttacttt ggttgggttg 60

gggggggcgg cgggctgttt tgttcctttt cttttttaag agttgggttt tcttttttaa 120

ttatccaaac agtgggcagc ttcctccccc acacccaagt atttgcacaa tatttgtgcg 180

gggtatgggg gtgggttttt aaatctcgtt tctcttggac aagcacaggg atctcgttct 240

cctcattttt tgggggtgtg tggggacttc tcaggtcgtg tccccagcct tctctgcagt 300

cccttctgcc ctgccgggcc cgtcgggagg cgccatggct cggatgaacc gcccggcccc 360

-continued

```
ggtggaggtg agctacaaac acatgcgctt cctcatcacc cacaacccca ccaacgccac    420
gctcagcacc ttcattgagg acctgaagaa gtacggggct accactgtgg tgcgtgtgtg    480
tgaagtgacc tatgacaaaa cgccgctgga gaaggatggc atcaccgttg tggactggcc    540
gtttgacgat ggggcgcccc cgcccggcaa ggtagtggaa gactggctga gcctggtgaa    600
ggccaagttc tgtgaggccc ccggcagctg cgtggctgtg cactgcgtgg cgggcctggg    660
ccgggctcca gtccttgtgg cgctggcgct tattgagagc gggatgaagt acgaggacgc    720
catccagttc atccgccaga agcgccgcgg agccatcaac agcaagcagc tcacctacct    780
ggagaaatac cggcccaaac agaggctgcg gttcaaagac ccacacacgc acaagacccg    840
gtgctgcgtt atgtagctca ggaccttggc tgggcctggt cgtcatgtag gtcaggacct    900
tggctggacc tggaggccct gcccagccct gctctgccca gcccagcagg ggctccaggc    960
cttggctggc cccacatcgc cttttcctcc ccgacacctc cgtgcacttg tgtccgagga   1020
gcgaggagcc cctcgggccc tgggtggcct ctgggccctt tctcctgtct ccgccactcc   1080
ctctggcggc gctggccgtg gctctgtctc tctgaggtgg gtcgggcgcc ctctgcccgc   1140
cccctcccac accagccagg ctggtctcct ctagcctgtt tgttgtgggg tgggggtata   1200
ttttgtaacc actgggcccc cagcccctct tttgcgaccc cttgtcctga cctgttctcg   1260
gcaccttaaa ttattagacc ccggggcagt caggtgctcc ggacacccga aggcaataaa   1320
acaggagccg tgaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa   1380
aaaaaaaaaa aaaaaa                                                   1396
```

```
<210> SEQ ID NO 799
<211> LENGTH: 173
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 799

Met Ala Arg Met Asn Arg Pro Ala Pro Val Glu Val Ser Tyr Lys His
1               5                   10                  15

Met Arg Phe Leu Ile Thr His Asn Pro Thr Asn Ala Thr Leu Ser Thr
            20                  25                  30

Phe Ile Glu Asp Leu Lys Lys Tyr Gly Ala Thr Thr Val Val Arg Val
        35                  40                  45

Cys Glu Val Thr Tyr Asp Lys Thr Pro Leu Glu Lys Asp Gly Ile Thr
    50                  55                  60

Val Val Asp Trp Pro Phe Asp Asp Gly Ala Pro Pro Pro Gly Lys Val
65                  70                  75                  80

Val Glu Asp Trp Leu Ser Leu Val Lys Ala Lys Phe Cys Glu Ala Pro
                85                  90                  95

Gly Ser Cys Val Ala Val His Cys Val Ala Gly Leu Gly Arg Ala Pro
            100                 105                 110

Val Leu Val Ala Leu Ala Leu Ile Glu Ser Gly Met Lys Tyr Glu Asp
        115                 120                 125

Ala Ile Gln Phe Ile Arg Gln Lys Arg Arg Gly Ala Ile Asn Ser Lys
    130                 135                 140

Gln Leu Thr Tyr Leu Glu Lys Tyr Arg Pro Lys Gln Arg Leu Arg Phe
145                 150                 155                 160

Lys Asp Pro His Thr His Lys Thr Arg Cys Cys Val Met
```

-continued

165 170

<210> SEQ ID NO 800
<211> LENGTH: 3925
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 800

agcggggctg cgcgaagtca tcgctgttcc agacagcgat gactcgagag cggtgggggt 60

ggcggcgcga tcggccgggc tgtaaccgtc gtctgtccgg gagcggctgg agcggcagcg 120

gcggccgggc acggcgcgag gtgacgccac agggcagcgg cggcagcgga ggcagcggcg 180

gcagcaggag acgcagcggc ggccgcagca gcagcagcaa gacggactcg tggagacgcg 240

ccgccgccgc cgccgccggg ccgggccggg tgtcgcgcgc cgaggctggg ggggagtcgt 300

cgccgccgcc gccaccgcta ccgccgccgc cgccgccgcc gaggtgactg aggagagagg 360

cgcctcctcg ctcccgccac cgccggactt caatgcccag tccccagctc gccagcgttt 420

ttcgttggaa tatacgttgc acatttatgg cgattctgag tgtgagggca gacttctgcc 480

aggctcagca cagcattttc gctgacaagt gagcttggag gttctatgtg ccataattaa 540

cattgccttg aagactcctg gacaccgaga ctggcctcag aaatagttgg cttttttttt 600

tttttaattg caagcatatt tcttttaatg actccagtaa aattaagcat caagtaaaca 660

agtggaaagt gacctacact tttaacttgt ctcactagtg cctaaatgta gtaaaggctg 720

cttaagtttt gtatgtagtt ggattttttg gagtccgaat atttccatct gcagaaattg 780

aggcccaaat tgaatttgga ttcaagtgga ttctaaatac tttgcttatc ttgaagagag 840

aagcttcata aggaataaac aagttgaata gagaaaacac tgattgataa taggcatttt 900

agtggtcttt ttaatgtttt ctgctgtgaa acatttcaag atttattgat tttttttttt 960

cactttcccc atcacactca cacgcacgct cacacttttt atttgccata atgaaccgtc 1020

cagcccctgt ggagatctcc tatgagaaca tgcgttttct gataactcac aaccctacca 1080

atgctactct caacaagttc acagaggaac ttaagaagta tggagtgacg actttggttc 1140

gagtttgtga tgctacatat gataaagctc cagttgaaaa agaaggaatc cacgttctag 1200

attggccatt tgatgatgga gctccacccc ctaatcagat agtagatgat tggttaaacc 1260

tgttaaaaac caaatttcgt gaagagccag gttgctgtgt tgcagtgcat tgtgttgcag 1320

gattgggaag ggcacctgtg ctggttgcac ttgctttgat tgaatgtgga atgaagtacg 1380

aagatgcagt tcagtttata agacaaaaaa gaaggggagc gttcaattcc aaacagctgc 1440

tttatttgga gaaataccga cctaagatgc gattacgctt cagagatacc aatgggcatt 1500

gctgtgttca gtagaaggaa atgtaaacga aggctgactt gattgtgcca tttagaggga 1560

actcttggta cctggaaatg tgaatctgga atattacctg tgtcatcaaa gtagtgatgg 1620

attcagtact cctcaaccac tctcctaatg attggaacaa aagcaaacaa aaaagaaatc 1680

tctctataaa atgaataaaa tgtttaagaa aagagaaaga gaaaaggaat taattcagtg 1740

aaggatgatt ttgctcctag ttttggagtt tgaatttctg ccaggattga attattttga 1800

aatctcctgt ctttttaaac tttttcaaaa taggtctcta aggaaaacca gcagaacatt 1860

aggcctgtgc aaaaccatct gtttggggag cacactcttc cattatgctt ggcacataga 1920

tctccctgtg gtgggatttt ttttttccct ttttttgtgg gggagggttg gtggtatatt 1980

-continued

```
tttcccctct tttttccttc ctctcctaca tctccctttt cccccgatcc aagttgtaga   2040
tggaatagaa gcccttgttg ctgtagatgt gcgtgcagtc tggcagcctt aagcccacct   2100
gggcactttt agataaaaaa aaaaaaaaac aaaaaacaac accaaaaaaa cagcagtgat   2160
atatatattc caggtggttt ttagtcttta ctgatgaaag ggtgttcatg ttagtttctt   2220
caaaacccta tctaatacta ggcaaagtag ccaagagcct tttgttttgt ttttattttg   2280
ataaattagt ggagaaatgg cattttaaga ggagtctctt ctcaacttac ctgagagtcg   2340
aattcttctc ttccctaacc aatgaagcta agtggttatc ccagaaactt gtcttctaaa   2400
agggaggact ccaggccatc aataaagatg tccaggcagt gagcgtactt tttacaccct   2460
gtagaattgt gggctgtagc gttactctga ttttctgtct agtatcagag aatgctggta   2520
gcttaaaatt tttattttag gacttgtact ctgaattttc aggaaccgtc aaaggagcag   2580
cagcaaattc acatattttc gacttgagaa atgcttgtgg tatgtgtttt ccaaactgcc   2640
ccctatatgt aaagttcagt ttaaccactg attgccttgt tattactagg ttttttgaga   2700
ttaaaaaaaa aaaatccctg gtttaaaacc aacaatgatg cctagtgagt atgtgtccac   2760
aggccataac agggtagaag agagacatcg tgcaacccaa tgagtagtga agggactgtg   2820
ttgcttgtga agcggtgtag tagcattttt gcagattctt ggctgggttt agtgtactga   2880
tctagaaaag ctgtttttct gctcctttgt ggaaggcagt tatgatcagg ctgcatggac   2940
aaagcaggta gaggggcacc atcaggggct cttgcactat tttcacctct aaatattacg   3000
tactcagtag tgccctgctt ctagggctct gaatacgggc ttaaagtcat cttgtcctgc   3060
tggaatttgc tgtgcagagc cataagcctc ccatttttgtt agcgtcagct aggccaatag  3120
gaacagaccg ggaccttgtc tcacactgat gatacctcac atgttgaccg gctatgtgaa   3180
ctgcctattt cctatgctgg agttttgatt tttaactaaa cgcaaatctg tagattctct   3240
cctctcccat cccagaaaac aaaacaaaat aatgcttttc gaaattgttt ctaggacttt   3300
aaaacataat ggtatatcca aaattcttta tttcagaatg caacaataga ttccattaat   3360
atagactcaa gatcaaaaca gcatacctgc taagctaaga tagatggtgt tgattccact   3420
gggttttgat caatacaata acaaaccttt ttcctttgac atactctgaa ttttgttgtt   3480
tggggggagg gggtgtgtgt gtgtgtgtgt gtgtgtgtgt gtattgtgtg tgtgtgtgtg   3540
tgcacgcgca gtgtccatca gtatcagtgc ctgcctgagt taggaaaatt acattcctgg   3600
ttctgtattg aggagaagga tgtataaagc aacatgaaac attagccctc cttttatttt   3660
aaagactaat gttaattgtt cttaaaactg gatttttttt ccttaaagca atttttttct   3720
tttcgattta atgaagtatt gctagctgaa gccagtttga catagagaga tgtcagattg   3780
atttgaaagg tgtgcagcct gatttaaaac caaaccctga accctttttaa agaacaataa  3840
aacatatttt acacgctcaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa   3900
aaaaaaaaaa aaaaaaaaaa aaaaa                                         3925
```

<210> SEQ ID NO 801
<211> LENGTH: 167
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 801

```
Met Asn Arg Pro Ala Pro Val Glu Ile Ser Tyr Glu Asn Met Arg Phe
1               5                   10                  15
```

-continued

```
Leu Ile Thr His Asn Pro Thr Asn Ala Thr Leu Asn Lys Phe Thr Glu
            20                  25                  30

Glu Leu Lys Lys Tyr Gly Val Thr Thr Leu Val Arg Val Cys Asp Ala
        35                  40                  45

Thr Tyr Asp Lys Ala Pro Val Glu Lys Glu Gly Ile His Val Leu Asp
    50                  55                  60

Trp Pro Phe Asp Asp Gly Ala Pro Pro Pro Asn Gln Ile Val Asp Asp
65                  70                  75                  80

Trp Leu Asn Leu Leu Lys Thr Lys Phe Arg Glu Glu Pro Gly Cys Cys
                85                  90                  95

Val Ala Val His Cys Val Ala Gly Leu Gly Arg Ala Pro Val Leu Val
            100                 105                 110

Ala Leu Ala Leu Ile Glu Cys Gly Met Lys Tyr Glu Asp Ala Val Gln
        115                 120                 125

Phe Ile Arg Gln Lys Arg Arg Gly Ala Phe Asn Ser Lys Gln Leu Leu
    130                 135                 140

Tyr Leu Glu Lys Tyr Arg Pro Lys Met Arg Leu Arg Phe Arg Asp Thr
145                 150                 155                 160

Asn Gly His Cys Cys Val Gln
                165
```

<210> SEQ ID NO 802
<211> LENGTH: 1785
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 802

```
atggcagcgg agtcagggga actaatcggg gcttgtgagt tcatgaaaga tcggttatat     60
tttgctactt taaggaatag accaaaaagc acagtaaata cccactattt ctccatcgat    120
gaggagctgg tctatgaaaa tttctatgca gattttggac cgctgaactt ggcaatggtg    180
tacagatatt gctgcaaact aaacaagaaa ctaaaatcat acagtttgtc aagaaagaaa    240
atagtgcact acacctgttt tgaccaacgg aaaagagcaa atgcagcatt tttgataggt    300
gcctatgcag taatctattt aaagaagaca ccagaagaag cctacagagc actcctgtct    360
ggctcaaacc ccccctatct tccattcagg gatgcttcct ttggaaattg cacttacaat    420
ctcaccattc tcgactgttt gcagggaatc agaaagggat tacaacatgg attttttgac    480
tttgagacat ttgatgtgga tgaatatgaa cattatgagc gagttgaaaa tggtgacttc    540
aactggattg ttccaggaaa atttttagca tttagtggac cacatcctaa aagcaaaatt    600
gagaatggtt atcctcttca cgcccctgaa gcctactttc cttatttcaa aaagcataat    660
gtgactgcag ttgtgaggct aaacaaaaag atttatgagg caaagcgctt cacagacgct    720
ggcttcgagc actatgacct cttcttcata gatggcagca cacccagtga caacatcgtg    780
cgaaggttcc tgaacatctg tgagaacacc gaaggggcca tcgccgttca ctgcaaagct    840
ggtcttggaa gaacagggac attgatagcc tgttatgtaa tgaaacacta caggtttaca    900
catgctgaaa taattgcttg gattagaata tgccggccag gctctattat aggaccccag    960
cagcacttcc tggaagaaaa acaagcatcg ttgtgggtcc aaggagacat tttccgatcc   1020
aaactgaaaa atcgaccatc cagtgaagga agtattaata aaattctttc tggcctagat   1080
gatatgtcta ttggtggaaa tctttcaaaa acacaaaaca tggaacgatt tggagaggat   1140
```

-continued

```
aacttagaag atgatgatgt ggaaatgaaa aatggtataa cccagggaga caaactacgt   1200
gccttaaaaa gtcagagaca gccacgtacc tcaccatcct gtgcatttag gtcagatgat   1260
acaaaaggac atccaagagc agtgtcccag cctttcagat taagttcatc cctgcaagga   1320
tctgcagtta ctttgaagac atcaaaaatg gcactgtccc cttcagcaac ggccaagagg   1380
atcaacagaa cttctttgtc ttcgggtgcc actgtaagaa gcttttccat aaactcccgg   1440
ctagccagtt ctctagggaa cttgaatgct gcaacagatg atccagagaa caaaaagacc   1500
tcctcatcct ctaaggcagg cttcacagcc agcccgttta ccaacctctt gaatggcagc   1560
tcccagccaa ctaccagaaa ttaccctgag ctcaacaata atcagtacaa cagaagcagc   1620
aacagcaacg gggcaacct gaacagcccc ccaggccccc acagcgccaa gacagaggag   1680
cacaccacca tcctccgacc ctcctacacc gggctttctt cttcttcagc gagattcctg   1740
agccgttcta tcccttccct tcagtctgaa tatgttcatt actaa                   1785
```

<210> SEQ ID NO 803
<211> LENGTH: 594
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 803

```
Met Ala Ala Glu Ser Gly Glu Leu Ile Gly Ala Cys Glu Phe Met Lys
1               5                   10                  15

Asp Arg Leu Tyr Phe Ala Thr Leu Arg Asn Arg Pro Lys Ser Thr Val
            20                  25                  30

Asn Thr His Tyr Phe Ser Ile Asp Glu Glu Leu Val Tyr Glu Asn Phe
        35                  40                  45

Tyr Ala Asp Phe Gly Pro Leu Asn Leu Ala Met Val Tyr Arg Tyr Cys
    50                  55                  60

Cys Lys Leu Asn Lys Lys Leu Lys Ser Tyr Ser Leu Ser Arg Lys Lys
65                  70                  75                  80

Ile Val His Tyr Thr Cys Phe Asp Gln Arg Lys Arg Ala Asn Ala Ala
                85                  90                  95

Phe Leu Ile Gly Ala Tyr Ala Val Ile Tyr Leu Lys Lys Thr Pro Glu
            100                 105                 110

Glu Ala Tyr Arg Ala Leu Leu Ser Gly Ser Asn Pro Pro Tyr Leu Pro
        115                 120                 125

Phe Arg Asp Ala Ser Phe Gly Asn Cys Thr Tyr Asn Leu Thr Ile Leu
    130                 135                 140

Asp Cys Leu Gln Gly Ile Arg Lys Gly Leu Gln His Gly Phe Phe Asp
145                 150                 155                 160

Phe Glu Thr Phe Asp Val Asp Glu Tyr Glu His Tyr Glu Arg Val Glu
                165                 170                 175

Asn Gly Asp Phe Asn Trp Ile Val Pro Gly Lys Phe Leu Ala Phe Ser
            180                 185                 190

Gly Pro His Pro Lys Ser Lys Ile Glu Asn Gly Tyr Pro Leu His Ala
        195                 200                 205

Pro Glu Ala Tyr Phe Pro Tyr Phe Lys Lys His Asn Val Thr Ala Val
    210                 215                 220

Val Arg Leu Asn Lys Lys Ile Tyr Glu Ala Lys Arg Phe Thr Asp Ala
225                 230                 235                 240
```

-continued

```
Gly Phe Glu His Tyr Asp Leu Phe Phe Ile Asp Gly Ser Thr Pro Ser
                245                 250                 255

Asp Asn Ile Val Arg Arg Phe Leu Asn Ile Cys Glu Asn Thr Glu Gly
            260                 265                 270

Ala Ile Ala Val His Cys Lys Ala Gly Leu Gly Arg Thr Gly Thr Leu
        275                 280                 285

Ile Ala Cys Tyr Val Met Lys His Tyr Arg Phe Thr His Ala Glu Ile
    290                 295                 300

Ile Ala Trp Ile Arg Ile Cys Arg Pro Gly Ser Ile Ile Gly Pro Gln
305                 310                 315                 320

Gln His Phe Leu Glu Glu Lys Gln Ala Ser Leu Trp Val Gln Gly Asp
                325                 330                 335

Ile Phe Arg Ser Lys Leu Lys Asn Arg Pro Ser Ser Glu Gly Ser Ile
            340                 345                 350

Asn Lys Ile Leu Ser Gly Leu Asp Asp Met Ser Ile Gly Gly Asn Leu
        355                 360                 365

Ser Lys Thr Gln Asn Met Glu Arg Phe Gly Glu Asp Asn Leu Glu Asp
    370                 375                 380

Asp Asp Val Glu Met Lys Asn Gly Ile Thr Gln Gly Asp Lys Leu Arg
385                 390                 395                 400

Ala Leu Lys Ser Gln Arg Gln Pro Arg Thr Ser Pro Ser Cys Ala Phe
                405                 410                 415

Arg Ser Asp Asp Thr Lys Gly His Pro Arg Ala Val Ser Gln Pro Phe
            420                 425                 430

Arg Leu Ser Ser Ser Leu Gln Gly Ser Ala Val Thr Leu Lys Thr Ser
        435                 440                 445

Lys Met Ala Leu Ser Pro Ser Ala Thr Ala Lys Arg Ile Asn Arg Thr
    450                 455                 460

Ser Leu Ser Ser Gly Ala Thr Val Arg Ser Phe Ser Ile Asn Ser Arg
465                 470                 475                 480

Leu Ala Ser Ser Leu Gly Asn Leu Asn Ala Ala Thr Asp Asp Pro Glu
                485                 490                 495

Asn Lys Lys Thr Ser Ser Ser Ser Lys Ala Gly Phe Thr Ala Ser Pro
            500                 505                 510

Phe Thr Asn Leu Leu Asn Gly Ser Ser Gln Pro Thr Thr Arg Asn Tyr
        515                 520                 525

Pro Glu Leu Asn Asn Asn Gln Tyr Asn Arg Ser Ser Asn Ser Asn Gly
    530                 535                 540

Gly Asn Leu Asn Ser Pro Pro Gly Pro His Ser Ala Lys Thr Glu Glu
545                 550                 555                 560

His Thr Thr Ile Leu Arg Pro Ser Tyr Thr Gly Leu Ser Ser Ser Ser
                565                 570                 575

Ala Arg Phe Leu Ser Arg Ser Ile Pro Ser Leu Gln Ser Glu Tyr Val
            580                 585                 590

His Tyr
```

<210> SEQ ID NO 804
<211> LENGTH: 2646
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 2300

-continued

<223> OTHER INFORMATION: n = A,T,C or G

<400> SEQUENCE: 804

atgaagcgga aaagcgagcg gcggtcgagc tgggccgccg cgccccccctg ctcgcggcgc 60
tgctcgtcga cctcgccggg tgtgaagaag atccgcagct ccacgcagca agacccgcgc 120
cgccgggacc cccaggacga cgtgtacctg gacatcaccg atcgcctttg ttttgccatt 180
ctctacagca gaccaaagag tgcatcaaat gtacattatt tcagcataga taatgaactt 240
gaatatgaga acttctacgc agattttgga ccactcaatc tggcaatggt ttacagatat 300
tgttgcaaga tcaataagaa attaaagtcc attacaatgt taaggaagaa aattgttcat 360
tttactggct ctgatcagag aaaacaagca aatgctgcct tccttgttgg atgctacatg 420
gttatatatt tggggagaac cccagaagaa gcatatagaa tattaatctt tggagagaca 480
tcctatattc ctttcagaga tgctgcctat ggaagttgca atttctacat tacacttctt 540
gactgttttc atgcagtaaa gaaggcaatg cagtatggct tccttaattt caactcattt 600
aaccttgatg aatatgaaca ctatgaaaaa gcagaaaatg gagatttaaa ttggataata 660
ccagaccgat ttattgcctt ctgtggacct cattcaagag ccagacttga aagtggttac 720
caccaacatt ctcctgagac ttatattcaa tattttaaga atcacaatgt tactaccatt 780
attcgtctga ataaaaggat gtatgatgcc aaacgcttta cggatgctgg cttcgatcac 840
catgatcttt tctttgcgga tggcagcacc cctactgatg ccattgtcaa agaattccta 900
gatatctgtg aaaatgctga gggtgccatt gcagtacatt gcaaagctgg ccttggtcgc 960
acgggcactc tgatagcctg ctacatcatg aagcattaca ggatgacagc agccgagacc 1020
attgcgtggg tcaggatctg cagacctggc tcggtgattg ggcctcagca gcagtttttg 1080
gtgatgaagc aaaccaacct ctggctggaa ggggactatt ttcgtcagaa gttaaagggg 1140
caggagaatg gacaacacag agcagccttc tccaaacttc tctctggcgt tgatgacatt 1200
tccataaatg gggtcgagaa tcaagatcag caagaacccg aaccgtacag tgatgatgac 1260
gaaatcaatg gagtgacaca aggtgataga cttcgggcct tgaaaagcag aagacaatcc 1320
aaaacaaacg ctattcctct cactctctcc atttcaagga ctaaaacagt cttgcgttaa 1380
gtaaaaacct gtgaccagag ctgaaggaag actctaggac tgaaaactgc aacagaaatt 1440
agcacaattt gaaaacaaaa caaaattgca aaagccttag ttgctttttc cacctaagaa 1500
gttgatcaat ggagaaaatg tccactggag tttgaataat gaactttgag tttgggtgca 1560
agcaaatgac tcagagaagg gtccagctct caagctgaat gacaaacatg ctgttgtaaa 1620
tttagtctca ggtgtaaata cccaagccct ctggtaccca gggagctggc tggtctgtgg 1680
tgcatgtgtg tccctgtgat ggcaatcatt gtagttgctg gccttcagaa gaattgagga 1740
tctgatggag gtttttttatg tatttatttt ctgttcacct tgtgaccctg tgtcaaaatt 1800
tataaagata caaaaggcat tactgaaatg gtactttctg taatttgata ctatttggct 1860
taatcatctt cacttgacta tttgtaatac tgttgtaatg ttaactctgt taagtaccca 1920
agctgcttgt cttccaccaa agagtgcttt attaacaaga atctgtgaaa atcacattta 1980
aacactgttg catgttgtaa gaccaggtgg taccttagta acctaaaact tgcaagagaa 2040
tattaatggt agctttagaa gactcaggag gagaaactga cttcagagtt ggaagatgtt 2100
gcaagtcgtt ccttttttctg tccttcaggg actgaagaac tgggaggctg cccattgttt 2160

-continued

```
ggttgccagt catacaaatt aaaatcatat ttccttccat gaatggaaga aacacactat   2220

tggtttttcc ccttggaaac agcaatccca aataatgtcg gcttacaaaa aaaaaagtta   2280

ccactttttt agagtccttn ccctgtaaca ttggattttt ttttccctta tgagatccac   2340

ctaaggccat tgacgtggcc tgcgatctca gtgacaatga tctgctttct ggatctcact   2400

gttgcctttg gttagggaac acagagtgct tctcccgcag ccctactgga acacagcaga   2460

gtctgtgcca tgaagcagtt acagaaacag aattgatgtg ctgctaaaaa aaaaaaaaaa   2520

aatggggccc gggggggcgt ccgccggccc tgcgggccgc cggtgaaata ccactactct   2580

gatcgttttt tcactgaccc ggtgaggcgg gggggcgagc cccgaggggc tctcgcttct   2640

ggcgcg                                                              2646
```

<210> SEQ ID NO 805
<211> LENGTH: 459
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 805

```
Met Lys Arg Lys Ser Glu Arg Arg Ser Ser Trp Ala Ala Ala Pro Pro
1               5                   10                  15

Cys Ser Arg Arg Cys Ser Ser Thr Ser Pro Gly Val Lys Lys Ile Arg
            20                  25                  30

Ser Ser Thr Gln Gln Asp Pro Arg Arg Arg Asp Pro Gln Asp Asp Val
        35                  40                  45

Tyr Leu Asp Ile Thr Asp Arg Leu Cys Phe Ala Ile Leu Tyr Ser Arg
    50                  55                  60

Pro Lys Ser Ala Ser Asn Val His Tyr Phe Ser Ile Asp Asn Glu Leu
65                  70                  75                  80

Glu Tyr Glu Asn Phe Tyr Ala Asp Phe Gly Pro Leu Asn Leu Ala Met
                85                  90                  95

Val Tyr Arg Tyr Cys Cys Lys Ile Asn Lys Lys Leu Lys Ser Ile Thr
            100                 105                 110

Met Leu Arg Lys Lys Ile Val His Phe Thr Gly Ser Asp Gln Arg Lys
        115                 120                 125

Gln Ala Asn Ala Ala Phe Leu Val Gly Cys Tyr Met Val Ile Tyr Leu
    130                 135                 140

Gly Arg Thr Pro Glu Glu Ala Tyr Arg Ile Leu Ile Phe Gly Glu Thr
145                 150                 155                 160

Ser Tyr Ile Pro Phe Arg Asp Ala Ala Tyr Gly Ser Cys Asn Phe Tyr
                165                 170                 175

Ile Thr Leu Leu Asp Cys Phe His Ala Val Lys Lys Ala Met Gln Tyr
            180                 185                 190

Gly Phe Leu Asn Phe Asn Ser Phe Asn Leu Asp Glu Tyr Glu His Tyr
        195                 200                 205

Glu Lys Ala Glu Asn Gly Asp Leu Asn Trp Ile Ile Pro Asp Arg Phe
    210                 215                 220

Ile Ala Phe Cys Gly Pro His Ser Arg Ala Arg Leu Glu Ser Gly Tyr
225                 230                 235                 240

His Gln His Ser Pro Glu Thr Tyr Ile Gln Tyr Phe Lys Asn His Asn
                245                 250                 255

Val Thr Thr Ile Ile Arg Leu Asn Lys Arg Met Tyr Asp Ala Lys Arg
            260                 265                 270
```

-continued

```
Phe Thr Asp Ala Gly Phe Asp His His Asp Leu Phe Phe Ala Asp Gly
        275                 280                 285

Ser Thr Pro Thr Asp Ala Ile Val Lys Glu Phe Leu Asp Ile Cys Glu
    290                 295                 300

Asn Ala Glu Gly Ala Ile Ala Val His Cys Lys Ala Gly Leu Gly Arg
305                 310                 315                 320

Thr Gly Thr Leu Ile Ala Cys Tyr Ile Met Lys His Tyr Arg Met Thr
                325                 330                 335

Ala Ala Glu Thr Ile Ala Trp Val Arg Ile Cys Arg Pro Gly Ser Val
            340                 345                 350

Ile Gly Pro Gln Gln Gln Phe Leu Val Met Lys Gln Thr Asn Leu Trp
        355                 360                 365

Leu Glu Gly Asp Tyr Phe Arg Gln Lys Leu Lys Gly Gln Glu Asn Gly
    370                 375                 380

Gln His Arg Ala Ala Phe Ser Lys Leu Leu Ser Gly Val Asp Asp Ile
385                 390                 395                 400

Ser Ile Asn Gly Val Glu Asn Gln Asp Gln Gln Glu Pro Glu Pro Tyr
                405                 410                 415

Ser Asp Asp Asp Glu Ile Asn Gly Val Thr Gln Gly Asp Arg Leu Arg
            420                 425                 430

Ala Leu Lys Ser Arg Arg Gln Ser Lys Thr Asn Ala Ile Pro Leu Thr
        435                 440                 445

Leu Ser Ile Ser Arg Thr Lys Thr Val Leu Arg
    450                 455
```

<210> SEQ ID NO 806
<211> LENGTH: 3415
<212> TYPE: DNA
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 806

```
ctcgcgggac acagagagag aagcaccggt gcttgtgcct ggcgcctgcc gagtccccga      60

cgctcgcccg tccgcgccgc tgcccgtggc ggccgcgtct ctgaaccgcg gggtcgtgtt     120

tgtgtttgac ccgcgggcgc tggcgcgtgg cacgggctga agcgtgcagc ggggcggggg     180

ccggcgcacg gaggcggagg aagacgagcg ggagtccggg caggcccggc ggcgccatgg     240

aactgggccc ggagcccccc caccgccgcc gcctgctctt cacttgcagc cccactcctg     300

cgccgcagcc cacggggaag gtgcagtttg gcgcgtcacg tgctggcgga ctgtcccctg     360

tcaccaacct gacggtcacc atggaccagc tggaagggct gggcagtgac tatgagaaac     420

caatggacgt gagaaatagc agcagtctac agagaatggg ctcctctgaa tcgactgatt     480

caggtttctg tctagattct cctgggccct tggacagtaa agaaaacctt gaaatttccc     540

tgaggagaat aaattgccta cctcagaagc tcttggggtg tagcccagcg ctaaagagga     600

gccattctga ttctctagac cacgacatct ttcaactcat tgaccaggat gaaaataaag     660

aaaatgaagc atttgaattt aaaaagccaa taagacctgc atctcgtggc tgcctgaatg     720

ctcacgttca cgaggaaagt aaggacccct ttacacacag gcagaattca gccccagctc     780

ggatgctgtc ttcaaatgaa agtgacatta gtgaatcagg aaatttcagt cctcttttta     840

caccccagtc acctgtgaaa gcgagtttgt ctgatgagga tgatggcttc atagaccttc     900

tggatggaga gaatctgaag aatgatgagg agaccccgtc gtgcatgtca agcctctgga     960
```

-continued

ccgctcccct tgtcatgaga agacctacaa accttgccga tcgatgtgga ctgtttgact 1020
ccccttcccc gtgcagctcc accagcagct gcagcactcg ggcagtgaag agagcagacc 1080
gatctcatga ggagtctcct cgaggtacaa agcggaggaa gagcagtgag gccagtccag 1140
tgaaggcaga tgttccggag ccaacgcagc ttccacacca gtctctctcc ctgacatctt 1200
tccccaaagg aaccattgag aacattttcc acagtgaccc aagagacctt ataggggatt 1260
tctccaaggg ttacctcttt catacggtct ctgggaagca tcaggatttg aaatatattt 1320
ctccagaaat tatggcatct gttttgaatg gcaagtttgc caatctcatt aaagagtttg 1380
ttatcattga ctgccgatac ccatatgaat atgaaggagg gcacatcaag ggtgccgtga 1440
acttgcacat ggaagaagag gttgaggagt tcttactcaa gaaacctatc gtgcccgctg 1500
acggcaagcg tgtcattgtc gtgttccact gtgagttctc ctctgagaga ggccctcgga 1560
tgtgccgata tgtacgggaa cgagataggc ttggcaatga ataccccaaa ctccactacc 1620
ctgagctgta tgtcctgaag gggggataca aggagttctt tttgaaatgc cagtctcact 1680
gtgaaccccc cagctaccga ccgatgcacc atgaagactt taaagaagac ctaaagaagt 1740
tccgcaccaa gagccggacc tgggcagggg agaagagcaa aagggagatg tacagtcgcc 1800
tgaagaagct ttgaggccaa atggcagtga cctgagcttc cctccgccct gtccctttgt 1860
ccctttgctg tagagcagta agcaaagggg ccagctatac ggcacctgga ccctggagaa 1920
aaacctgggc cttccatgcc ttgaacctcc tacactccca ggttggagcc caggcatcct 1980
gccgtcacac tcttctgtga gagtccttcc ctgtcaggac tgtctgccaa agctggacaa 2040
gctcggcaca ggctggcaca ggctcgagtc tagtctggaa cgccacgtca ggctgctccg 2100
actaagcatc ccctgaagaa gtgcccaggc ctctcatgag gggagagaag ccactgaagt 2160
gctgctggcc aaataccaaa gataggctgg aaggggagag gtcctcatgg atgactcttt 2220
aatttattca gcctcatcaa ttattttatt attgttttaa ttcctcaaga cttttacttt 2280
actgcttcaa agtcaaaata ctgccattct aggtagagtt ttatcatcct aggaactacc 2340
tctactttta atttaaaaaa aaaacatggg gcagggataa gaaaaaaggc aaacctgtta 2400
agtgtgggca gcgcaaggaa ctcagtcacc cctaggaggc gctgtagact ggtattgctg 2460
ctattcaaag tcaaggactg agatgctggt cagagcctgc accaaccaga tccaggcttg 2520
gctacaggac ataagctaac cttcccagac ctacttctgc cctttgtgag ttcctttggg 2580
gagagtcttg tctgtactcc tggtcccagg tccccgtgac agtgactggt gtgggagttg 2640
caggaaggca catcaagcca cccccaggcc agtactggaa tgttgaagtg taccccaagg 2700
tgggagtggg gaggcatgga aaagtggagt ccacagagta agggaggagc atgcccactg 2760
aatgtccttt agaaaaaaaa aaaagtcatt ttatgagtca gagtatccaa tcagtgttgg 2820
gtgggcacct aagcttgagc agggggcggg aagcccgggc tgttacagac gactgtagaa 2880
tttctcagga gggcgtagta aattttgaag tcaaaagttc tgggtttcat catgttttaa 2940
ttgagggaca gagtggtgaa acacatcagt taccccctaa tctaaccccg tggaagtgag 3000
gctctgggga atgcctccca tctaaggagc tggcccgttt tgattctgtc agtgtcctcg 3060
ggcaccagcc tccctgccat ctgtgctcca ttggggtcat gccaggtttt tcttaggaag 3120
agtctcccct cttaacctct gctttctatt ctgggggtgg ggagggaatc aatgatattg 3180
aagatggcta gttgctttgt taagggtttg agtttgcatt tggctataaa acaaatcttg 3240

-continued

ttaaaaatat gtggagagca agggaatgag cagcctcttc ttcggtgtgt tgaagtatgt 3300

cctagttttc ccctggtctg gtttgtagag attctgttag ttgaatgcct tcaaggagaa 3360

tgaatggctt tcagattgta ccagcttagc tagcattgtt aaccagctgc tgcag 3415

<210> SEQ ID NO 807
<211> LENGTH: 525
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 807

Met Glu Leu Gly Pro Glu Pro Pro His Arg Arg Arg Leu Leu Phe Thr
1 5 10 15

Cys Ser Pro Thr Pro Ala Pro Gln Pro Thr Gly Lys Val Gln Phe Gly
20 25 30

Ala Ser Arg Ala Gly Gly Leu Ser Pro Val Thr Asn Leu Thr Val Thr
35 40 45

Met Asp Gln Leu Glu Gly Leu Gly Ser Asp Tyr Glu Lys Pro Met Asp
50 55 60

Val Arg Asn Ser Ser Ser Leu Gln Arg Met Gly Ser Ser Glu Ser Thr
65 70 75 80

Asp Ser Gly Phe Cys Leu Asp Ser Pro Gly Pro Leu Asp Ser Lys Glu
85 90 95

Asn Leu Glu Ile Ser Leu Arg Arg Ile Asn Cys Leu Pro Gln Lys Leu
100 105 110

Leu Gly Cys Ser Pro Ala Leu Lys Arg Ser His Ser Asp Ser Leu Asp
115 120 125

His Asp Ile Phe Gln Leu Ile Asp Gln Asp Glu Asn Lys Glu Asn Glu
130 135 140

Ala Phe Glu Phe Lys Lys Pro Ile Arg Pro Ala Ser Arg Gly Cys Leu
145 150 155 160

Asn Ala His Val His Glu Glu Ser Lys Asp Pro Phe Thr His Arg Gln
165 170 175

Asn Ser Ala Pro Ala Arg Met Leu Ser Ser Asn Glu Ser Asp Ile Ser
180 185 190

Glu Ser Gly Asn Phe Ser Pro Leu Phe Thr Pro Gln Ser Pro Val Lys
195 200 205

Ala Ser Leu Ser Asp Glu Asp Asp Gly Phe Ile Asp Leu Leu Asp Gly
210 215 220

Glu Asn Leu Lys Asn Asp Glu Glu Thr Pro Ser Cys Met Ser Ser Leu
225 230 235 240

Trp Thr Ala Pro Leu Val Met Arg Arg Pro Thr Asn Leu Ala Asp Arg
245 250 255

Cys Gly Leu Phe Asp Ser Pro Ser Pro Cys Ser Ser Thr Ser Ser Cys
260 265 270

Ser Thr Arg Ala Val Lys Arg Ala Asp Arg Ser His Glu Glu Ser Pro
275 280 285

Arg Gly Thr Lys Arg Arg Lys Ser Ser Glu Ala Ser Pro Val Lys Ala
290 295 300

Asp Val Pro Glu Pro Thr Gln Leu Pro His Gln Ser Leu Ser Leu Thr
305 310 315 320

Ser Phe Pro Lys Gly Thr Ile Glu Asn Ile Phe His Ser Asp Pro Arg

-continued

```
                325                 330                 335

Asp Leu Ile Gly Asp Phe Ser Lys Gly Tyr Leu Phe His Thr Val Ser
            340                 345                 350

Gly Lys His Gln Asp Leu Lys Tyr Ile Ser Pro Glu Ile Met Ala Ser
        355                 360                 365

Val Leu Asn Gly Lys Phe Ala Asn Leu Ile Lys Glu Phe Val Ile Ile
    370                 375                 380

Asp Cys Arg Tyr Pro Tyr Glu Tyr Glu Gly Gly His Ile Lys Gly Ala
385                 390                 395                 400

Val Asn Leu His Met Glu Glu Glu Val Glu Glu Phe Leu Leu Lys Lys
                405                 410                 415

Pro Ile Val Pro Ala Asp Gly Lys Arg Val Ile Val Val Phe His Cys
            420                 425                 430

Glu Phe Ser Ser Glu Arg Gly Pro Arg Met Cys Arg Tyr Val Arg Glu
        435                 440                 445

Arg Asp Arg Leu Gly Asn Glu Tyr Pro Lys Leu His Tyr Pro Glu Leu
    450                 455                 460

Tyr Val Leu Lys Gly Gly Tyr Lys Glu Phe Phe Leu Lys Cys Gln Ser
465                 470                 475                 480

His Cys Glu Pro Pro Ser Tyr Arg Pro Met His His Glu Asp Phe Lys
                485                 490                 495

Glu Asp Leu Lys Lys Phe Arg Thr Lys Ser Arg Thr Trp Ala Gly Glu
            500                 505                 510

Lys Ser Lys Arg Glu Met Tyr Ser Arg Leu Lys Lys Leu
        515                 520                 525
```

<210> SEQ ID NO 808
<211> LENGTH: 31868
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 808

```
ccagggtctg tgagccctcc agagttgggc cctggtggtc gagtccagtc ctgggggtca     60

ttgcattccc tccctcatta taaaatgggg cctggaggcc cggggcggaa gaaaggggtc    120

cacaatactg cacggttaga ggccgagcca aggctggatc cggccagacc tccacaggtc    180

ttccttagcc tccacattgc ctcagagtgt ggggcgcccg gctgggggcg aggtagcgga    240

ggcccaaagg gggccgaagc taactggacg gcagctcgcg atgggaacta cgcttcccag    300

catgcgacgg ggcaaagggg cctttcagcc gcgagcagcg cctcgcaggt tctgctggga    360

gttttcattg acctctgctc cccctctcat tttgatcccc gctcttctgc tctgggctcc    420

gccccttct gagagccgat gacctggcag agtcccgcga gccgctttct tcttcccctc    480

tcattggccc agcctagctg ccattcggtt gagaggagga gaagttgctt actgattggt    540

ggattccgtt tggcgccaac taggaaaggg gggcggggca gcagctggcc ccactgagcc    600

gctattaccg cgaaaggccg gcctggctgc gacagcctgg gtaagaggtg taggtcggct    660

tggttttctg ctacccggag ctgggcaagc gggtgggaga acagcgaaga cagcgtgagc    720

ctgggccgtt gcctcgaggc tctcgcccgg cttctcttgc cgacccgcca cgtttgtttg    780

gatttaatct tcaggttgcc ggcgcccgcc cgcccgctgg cctcgcggtg tgagagggaa    840

gcacccgtgc ctgtggctgg tggctggcgc ctggagggtc cgcacacccg cccggccgcg    900
```

-continued

```
ccgcttgccc gcggcagccg cgtccctgaa ccgcggagtc gtgtttgtgt ttgacccgcg    960
ggcgccggtg gcgcgcggcc gaggccggtg tcggcggggc ggggcggtcg cggcggaggc   1020
agaggaagag ggagcgggag ctctgcgagg ccgggcgccg ccatggaact gggcccggag   1080
cccccgcacc gccgccgcct gctcttcgcc tgcagccccc ctcccgcgtc gcagcccgtc   1140
gtgaaggcgc tatttggcgc ttcagccgcc gggggactgt cgcctgtcac caacctgacc   1200
gtcactatgg accagctgca gggtctgggc aggtaaggag agaccggcgg gcggtgctcc   1260
gggcccctgg cctcggtgtc ggcctcggag agatcaggcc aggaaacgga ccgggagaag   1320
ggcgagaccc gtccgtccgg gttcgccgct cggggacagc cgggctaggg cctgccatgt   1380
gcacccccgc ccgggcggaa tgttgggcgg gagaggccgt cgggaccttc caggggaaga   1440
ggtggagatc cttgggccta agcccgagcc aggcccacct tcaccccttt cggattgctc   1500
cgtactctcc ttctatctct atccctggaa gctctttgga atctaccccc gcggggaaaa   1560
tcaggctctt ctaggcactc actttcaccc tttgctaaac catcctcagg atcttcgttt   1620
gctgtgatct ttgttccttc tcaacaaagg accatggcat tttctttcct ggcgtttatg   1680
taaaatcatc tcagtccctc gccctgtgca cattcctgat gtccactctg ctgctttcct   1740
aaggccaggt ctttttaccc aactttcaga aagcttcctg ggcttttcct gatagcaaaa   1800
aatgcatccc acggtgtttc ccgcggaaga gctactttcc cttcaatctc tggcatcccg   1860
tttgctaagc acatgtcttg tgcgtttccc aacttctgaa aagcagaaag tgtcctgttc   1920
aactttcatc ccgactctgt ctcagtactt agaacacatg cttttatttt aggaaatacc   1980
ccaacatttg ccatagccat cataacctgc aatgtggtcc aaggccatgc ccacccactc   2040
ctttttctc ctttgcccaa gtgctaattg ggtgttcaga gtggcaaagt gggatctttg   2100
ccacttgtgg tgtggcctag aaatggtttc tggcagcctg gctgcttctt aatctcatgg   2160
cctatctcct gcatgtgacc ttttaattat atcctataaa tcattcatgg tttatttctg   2220
ttggtttcag tgattatgag caaccactgg aggtgaagaa caacagtaat ctgcagagaa   2280
tgggctcctc cgagtcaaca gattcaggta tttaagtctg ctgtgtgggg agcaatactc   2340
tgaatttcct gaaacatgcc ttttcaccca ggaggttagt tttggtgaga acttgaggaa   2400
gtcatggcat tgggtgataa actttttttt tttttttttt gagacagagt ttcgctcttg   2460
tcgcccaggc tggagtgcaa tggcatgatc tcagctcact gtaacctcca cctcccgggt   2520
tcaagtgatt ctcctgcctc agcctcccga gtagctggga ttacaggcat gcaccaccac   2580
acccggctaa ttttgtattt tttagtagag acagggtttc ttcatgttgg tcagcctggt   2640
ctcgaattca caacctcagg tgatccgcct tcctcggcct cccaaagtgc tgggattaca   2700
gcgtgagcca ccgcgcccgg tgataaacat tattgagaac aatacaaagg agcccttgtg   2760
gctgtttatg aagaaaggaa ataggtttca aatgtctata aggaggtggt gtgtgcttgc   2820
ccttgaagga tgggtagtaa agtataattc accataccac tgtaagtggc attcaggcaa   2880
tcttactggt taaaatacag gatcaaatga ttggaagtac agtgtcatga aatcatttca   2940
gtgatgctgc tatggaggaa attgccagtg catttcattc ttcatgaatt catattactg   3000
cagagtttat ctgtatttgt acagataaga ccattggtgc aaagatcctt taggttaaag   3060
gaactgcaag agcaagtctt aaaatgtaat gtaggcttct gtatgtagaa tgtaatttaa   3120
tatagaactg ggataggat tatcacttgt agcagtgtgg tgagcaaggg ctgtaacacc   3180
```

-continued

```
tcactttcca taaggctgta tagacacatg agctatgggt gggtgggtct gcgtcatctg   3240
agctggcata ttagtaatgc tgaacagtgt ctccttcacc ctgctgcctt ttgtgagatg   3300
gacaccttgg tgtcattttg ttaaaggcag caagtgcttg ccttgcatgt catgaccagt   3360
agttgtattt ttctttttca ttttgagaca aggtctcaac tctgtcaccc aggcaacctc   3420
tgcctcccag atttagagaa ttcttctgcc tcagcttccc tagtagctgg aattacaggt   3480
gtgggtctca aacttctgat ctcaggtgat ccacccactt cgacctccca aagtgctgag   3540
attacagggg tgagctactg cacctggcct tttttttttt tttttttga gacagggtct   3600
tgctctgttt tccaggtctc aggctggtgt acagtggcac aatcagggtt cactgtagct   3660
tcaactttcc gagctcaagc gaacctccca ctccagcctc cccaagtagc tgggactaca   3720
gacacgtgcc accacaccca actaattttt tttttaactt tggtagagtc aagtctcact   3780
atgttgcccc aggcttgttt tgaacttgtg gcctcaagcc atcagcctgc ctcagcctcc   3840
caaagtattg gaatcacagg tgtgagccac tgtgcctggt cagtagttgt atttttcaag   3900
cctcatatga tttgacaagg taaaacgttt aaaaactatg ccaagaaaat ctggtaccta   3960
ttgtttctat ggttggaact cacagaaagt tatctgggtc acctctatct gaataaccaa   4020
acaatcatga tatgaatcag ttttattcac cctcttctca aatcctattt cccttgatgt   4080
tatttgcaaa taggagaaaa aacagtaaat acgctatgaa aatattaaga cttgtacaaa   4140
catgacattt ttgacaatct aatagacgta acatcaattc tggcaaaagt acaagccatg   4200
aatatttgtt gcctgactta aataaagacc caggtacttt gtttagtgtt ttaattttaa   4260
tgacaaaatg gtgttttttg tattatttct tgagaggttt tttttttccc cataggtttc   4320
tgtctagatt ctcctgggcc attggacagt aaagaaaagt atgtattcac tgctttcaaa   4380
tgtttatatg tagaaaaaac gtgtctaaac ttaaatacct tatttaaatg aagttcttca   4440
tacactggac tggagccctg gatttacctg tgcctagaaa cactctggaa tctcaagaat   4500
gaatagtttg tgtcagcaat tttcagaact ttttctcctt tattttagct ataattttgg   4560
tgttctgttt gggtcttacc caaaccctta ctctgtgctc tgacatcata gccttacaag   4620
aacatgtggg gttttttttgt ttgtttgttt gtttgtttct gaggcagagt ctcactctgc   4680
cgcccaggct agagtgcagt ggctcactgc aacctccacc tcccaggttc aactgattct   4740
cctgcctcag cctcccgagt agctgggact acaggagtgc accaccacac ccggttaatt   4800
tttgtatttt tagtagacac gaggtttcat cgtgttggcc aggccaggag ttcgagactc   4860
ctgagactcc tgacctcgtg atataccctc ctcggcctcc catagtgccc atagtgctgg   4920
gattacatgt gtgagccacc gcgcccagcc gaatgtgtgt attttaagaa aagacatggg   4980
tttgtttttt ttcacctaga agtctagtgt tgggggtgct cctgagaaca ggacagcttc   5040
agaaatttat tctcccatct cttgcctaaa atgggagtct gtgactgtac ccccaagcta   5100
caggctaaac actatctctc tgctaatatg aatacctctt tacttgtttt attctcatta   5160
tttaccttct gatctcatta cagccttgaa aatcctatga gaagaataca ttccctacct   5220
gtaagttagt tccttgttta ttttgagcta atacctgtta tctgttcctt agctaccagc   5280
atgaccttga taggagactt aatttagaga gtaaaaactg cttttcttta gtctcttttg   5340
agacaaggtc ttgctctgtc acccaggctg gagtgcagtg gcaaaatctt ggctcactgc   5400
aacctctgct tccctggctc aagtgatcct cccacctcaa cttccagagg agctgggact   5460
```

-continued

```
atatataggt gcacaccatc acacccggct gatttttgta ttttttgtag acatgggggt   5520
ctcactctgt tgcccaggct ggtttcaaac tcctgcctcg gcctcccaaa gtgctaggat   5580
tataggcaag agccactatt cccagccttt ccttagtctc ataagtactc aagatgttcg   5640
gtctggagga atttgtcctc catctctaac agagctttat taaaccagga agggttcttt   5700
tgatgcaaat gttatttggg gattgatttg gcactgtaat ccctgttgag gggggcactg   5760
tagaccttgt gaggttagct gtaaacccat ggaatggaaa cttctatgct gtgtcctggc   5820
tgtgtcctgg caaggtggat tcctggccac atttactggt tacctactgc agtgtgtgag   5880
gagctagtct accccctatt caaccccccct tttttttctt ttgagaactg aattttccac   5940
tgaaatttta tttgtgtgcc ttaattattt tccttttttta ctttgatagc agaagctgtt   6000
gggatgtagt ccagctctga agaggagcca ttctgattct cttgaccatg acatctttca   6060
gctcatcgac ccagatgaga acaaggaaaa tgtgagtgtg acttcaatgt actaacctga   6120
ggcagaggtg aaacccacag gctgtcagtg gcttagaagt ggctgggctg ctttctggga   6180
gactaaactt ggccattatg tgcggtcttt ggagtcagag aattctgggt ttggatcctg   6240
cttactagct gtgtgatgtt ggcaaattct tctcttttta atctttgctc ttttgtaaaa   6300
taggagtagt aataccttttt agggatgttg tggcaataag taagattatg tatgaagtat   6360
tgaaaatggt gcctgacagg cgggagttgc tcagattgta gctcacagga gtaattacat   6420
gtacaaatag tgtttgtctg gaaggctctg cccaggctgt ccttgcctct ttgctctgac   6480
cctgaaatga attgtaagct taggaaaagg ctctttggca gtatttgtaa gaacggggtc   6540
aacaagttct agggaatctc agagtaactg aaatggtggc ttggaaggtg aatttctggt   6600
tatctaccct gaatgagcct ggcttttttg ttcttttaga cattgcacag agtaggcatc   6660
caaaacagtt gtaatttttt cagccttctc tctactgtat ttctatatat ttactttctc   6720
cccttttcca ttcctgctga aatgccacag ttcataatcc ttgtccttac aatcttcagg   6780
tttagtgtga aacccactgc ctccatgaac tctcctgatg atttctttat caacctggga   6840
agtgtcatga gaaagaatat ggaaatgagt tccagccttg agtgcttgga acagtgtatc   6900
agcctctagt gctttttacc agggcaaagc tgtactgatg cctggttttc ccccgaaata   6960
gcttctcagg gttgcttgcc tggagctttg ttttaaaggg aaaacagcat agatggtggt   7020
taaagagctc tcactctgga gttgtcctgt tttacttcaa tattttgggc acattcctta   7080
acctgcaagg tctagttttc tcacctgtaa aatcgggaca atcaggttgt atttgatggt   7140
taaaaagtta atgcacagaa agtacttagt actgtgttgg catagaaatc actgtaatat   7200
tagccattac tgcttttttt ttttttttga ttcttcatgg ttttatttct ctcagaactt   7260
taaaatgtga acattctata attcagccag tctttacttc caggtaactt ctttattggg   7320
tcgcaatacc cttttaaata acatgctctt gttccggaag gtagttagcc gttcatcact   7380
ctttctgtct aaataacatc cagtgacaaa tcccataggg acaagaacat gaacccagtg   7440
gtcccgcaca atctgaagta agttcaccat gatagctgca gcctcagtgc cgaccgcgac   7500
cccgagacca agggcaacgg ggaactcagc cacccacgcc agtgctagcc attactgctt   7560
ttaatactta attatgagtt cttaagtgaa tattttaggg caagggacct gtgagtgttt   7620
tcattccata aagtggaatc atacgatatg tattctatca tatctggctt taaaaaacat   7680
tatgcttgta agaatcgtcc acgttgagta aaccagtagg gcttttttttt ttttcattgc   7740
```

-continued

```
ttttagtata gaatagaata attataagta gaaaaaatta tctccagttg gtaggaagag   7800
gtggaaaata tgaagccctg aaacagatgg acctagatgg aaaattttga caactattca   7860
caggcaatta tattagattt tatttatttt tgctttagat ttcagaaagt ttgtgattta   7920
gcttattcat cgtgttttgt atatttggca gatacttgtg ttaggaaata cagtatgaca   7980
agaaaaagat gttactttag aagttagctg ttgacagagc cttggaaatt cttagttacg   8040
cagatctatt gttggaatta ggtgaaactt taatttggag gcagaaaggt gatcagtgcc   8100
cactttcttg cctctttcac ccaagctgag gcctgagttg gttttcatgg acagtagatg   8160
tctcaactat gtcttgcagg aagcctttga gtttaagaag ccagtaagac ctgtatctcg   8220
tggctgcctg cactctcatg gactccagga gggtaaagat ctcttcacac agaggcagaa   8280
ctctgcccca gctcggatgg tatgtgctct ggctttcata ggggaattcc tgacaggaag   8340
aaaggattaa aacaccttct ttcatccaga attgaaggca ctacagtagc actagctgtt   8400
tttaccttga ctttgtcttt tgaaattaga ctgtcaagca ttcttggtgg ttttgctgtg   8460
tctggagaac agacaggcgg tagtaagagt gggagatggt gtttgaaata ttggagttgt   8520
gcaaggaata atcgaccttg ttaatctggg aaatcctggg tcatcctaaa tgtatgtatg   8580
gtgggattat atctcttcct atttttttct ttttttcttt tttttttttt aagggctttc   8640
tggcaaaaac cggaaagcct gcgagacaaa ttttaaaaga gccgtaacac tagatctctt   8700
acttaaaact tggtctttac tatgcattct aagtgcttct caaggaactg gagagagagc   8760
acttgacttc ttccaaaggt ggtttgttaa gaacagttat gggccaggtg ccatggctca   8820
tgcctataat ctcagcactt tggaaaactg agacaggagg atcgcttgag tcctgggcag   8880
catagtgaga ccacatctct attttttttta tttttaattt ttttaaagaa ctattatgga   8940
ttacagtgaa tgctcaggct gctgggcagt tttggggtca ctcgtttcag ggctatgtat   9000
ttgccagaca gggactgtca ttacttccaa agtttcctgc tgttggtctg aattggatta   9060
actgatagca tctatagatt ggagaggccc aacttgagca agatgaccac atttggcttc   9120
caggtttacc taggatctta aatctgaaaa tatacctttc caatctgctt tgtgttctga   9180
ggttcagcca gtattgttac tctgtctatc atttgcacaa agcactttta tatgttcttg   9240
tcttgtgaag tggagttaat ccacttgaac agattaggaa agtaccacta gagaggtttg   9300
gatgatttac caaaggatat acataagtag gaaaccagga tttaatgatc tctggctggg   9360
tgtggtggct catggctgta atcccagcac tttgggaggc cgaggtggga ggatcacatg   9420
aggtcaggag ttcaagacag cctgggtaac atagtgagac cctatctgta caaagttaaa   9480
aaataagctg agtgtggtgg cttgttccta tagtcccaat tagtcgggag gctgaagtgg   9540
gaggattgct tgagcccaag aattcgaggc tgcagtgagc tatgatcaga ccactgtact   9600
ccagcctgga caatgaatcc ctgtctcaat aaacaatctc taaattccca agtacacagt   9660
aatgctttaa gagttgggta tcagaaacaa tatatttggt gtgtgcttag cgactagagc   9720
ctgtatcaca tctgcaccta ggaatcccag aacatacctc acaaatgttg gtgtgatata   9780
gtgtgagctt tcgtgataaa ggtactgccc cataataaag ttatctaccc ctaggctaaa   9840
aaaatttgcc atttcccaga gtgtgcactt ggggaggact agagactgct cagaacttgt   9900
tttatattgt gaagcaaagc taatgccaga accagaatag gccagctgca gagaggattc   9960
cttggtgcat gatccactca gaaaatcaga gggccactta actaacaaca gattcatacc  10020
```

-continued

```
ccaaagagat ttggatttta aggattactt gtaggtcata acattgggac taccctgcta  10080
ctgtgaaaaa aataactcta aacttttttt tctggatttg aaagtgccta gaaaataaat  10140
aattcagtat tagcagtgtt tgtattggta ggatttatct tcaggtgtgg atttatgagc  10200
agtttttcta aattttctat attccatgtt tagaggtttc ccaaaaaaag tatgactata  10260
ggttggctaa tgaggtaccg ccattttaat tccaggaatg gttttctcct agggagatag  10320
tgtggtgaga agagtttgga ttttggagtc aaacagatga ggtgtataat cttggtaacc  10380
tcttaagcac cagtttcctg atttgctaaa gaaaaaggaa aggattagct acctttagtg  10440
tattgtgagg cttctattgg attatatgta taaagattat atcgtagtat ctggcacaca  10500
gtaattcctc gtttaaaaac tgagcattta aatcccaagt ctaaagaacg aggatagatt  10560
ttatagaagg aagttaccct ttattccccc ctgtcagaac tggagttata tataagtgca  10620
ttctctaggc cattttatgg tctttataga gatttgcatc tgcttgccct aactcatttc  10680
agcagattct catactgtca cagtcctcaa tgctgatcgg tgttcactga tgcaaccttc  10740
taataaaaga aaccttgttc ttcacaagag gctatctcta gtacccatta taaggtgaat  10800
tgcttctggc aagagttctc tgtaaaggct actgactact cagggctgtg tgtggtcatc  10860
aaatcattta taatcttggg atacattttc atataatcag taatggctaa aattttgctt  10920
tgtataacaa gtataacata gtatgttttc atcataaaaa ctagtgaccc attcaaggaa  10980
atgaaagtgg atcagagctc tcattattaa tccatgaatt ttgtcttaca gctttcctca  11040
aatgaaagag atagcagtga accagggaat ttcattcctc tttttacacc ccagtcacct  11100
gtgacagcca ctttgtctga tgaggatgat ggcttcgtgg accttctcga tggagagaat  11160
ctgaaggtac cgtgtgtgtg tgtgtgtgtt cctatttgtt ctactaatta attacctctg  11220
gagaaggcat gtgatgtgaa aaagaacagc aacagcagtt ccccggggct tgcttagctg  11280
atatttttgt tcatttggtg ataattcatt taataatagg gctaccagcc ttattaaatc  11340
ctttggattg ggggatgggg gcatcaaaag aagacacatg atccttctta ccctgaagga  11400
gctaactaac aatctatatg caagaaacat gaaatcagaa cggtatagga tggtacatta  11460
aggatacttc ctatccaggc ctatctggta ttgttcaggg agaaacacac ctaaagcact  11520
taaacaaatg ttagtctcta caggctcttt ttaaaatcag tttttctgtt tctttagaat  11580
gaggaggaga ccccctcgtg catggcaagc ctctggacag ctcctctcgt catgagaact  11640
acaaaccttg tgagttgttc tagtgtgtct ggaggaagcc ctgcgtgatt ggggcactgg  11700
acagagtagt ccttagttga gtatagccaa agattgaaat gcatgatagg atttggggat  11760
ctgggtttta ggcctcactt tggctaccta caaattatga cctttagtca tgatatctct  11820
ctgcatgtct cctgatgtat aaggagtgtg ggttaggagg gaccatggtc attcctagct  11880
tttaccatct agtcagtttg aaaaaagcct atctgaagcc tttagcctga atactttact  11940
ttttttaggt tattctcatt tatattccac agaacctagc ataaaattag acaagcaaca  12000
gtaactcaca ggattttttt tgtttctgtg tttttttgttt ttttttttga gacggagtct  12060
cgctcttgtt gcccattcta gagtgcagtg gcacaatctt ggctcactgc aacctctgcc  12120
tcccaggttc aagtgattct cctgcctcag cctcccgagt agctgggatt acaggtgcct  12180
gccaccacac ccagctaatc tttgtatttt cagtagagac ggggttacat tatgttggcc  12240
aggctagtct cgatctcctg acctcaggtg atctgcccac ctcgacctcc caaagtgctg  12300
```

-continued

```
ggattacagg catgagccac tgtgcctggc tggatgtttt ttgttgctgt tttgtttgtt  12360
tgttttgttt tgttttgttt ttcattgaaa atacctacct gaggctgggt attttcaggt  12420
agatattttc gattttaaaa attatatata atatatattt tatatatata tatatatata  12480
tatactcaca cacacacata cacagtatag atttgtgttt ccatcaggga tactttcaaa  12540
cagaaagcat agtatatgta gatacagaat ctatagtgta tgtgtgtata tatattcaaa  12600
atcacatata tatacacaca catacacatg ctatagattc gtgttttcat catagggata  12660
ctttcaaagt tagaagcaag aatgtgccta tacagatata tacacctgta tgttcagaga  12720
tatacaccct taggtacaca tctaaattga taagttcata ttttttaatt ctcaataacc  12780
tgaactataa ttacagaaca aatgagctac agttttttttg tttgtttgtt ttgttttgtt  12840
tttgagacag agtctccctc tgttgcccag gctggggtgc agtgcagtgg tgtagtcttg  12900
acttgctgca acctctgcct cctgggttca agtgcttctt ctggctcagc cttctgagta  12960
gctgggacta caggcttacg ccaccactcc cggctaattt ttttggtatt tttagtagag  13020
acgaggtttc actatgttgg ccaggctggt ctcgaactct tgacctcagg tgatccatcc  13080
acctgcctcc caaagtgctg ggattacagg catgagccac cgtgcccagc caagctacag  13140
ttttgagatc acttatggat tatagtacat tcatgtgcat agcttttgag aaaatatgtt  13200
aacgcagtac aaatgttatg agtaaattta ccaggtcatt ttgataggca taaaattaca  13260
taaaatgtga gaacactttg gaagtggctg ttagttttat agatttgatt ttagagtcat  13320
aagcatgggc ttgtaagctc ttaatagtgg ggaaatgtct tccacaaatg gttattacaa  13380
actaaccatt gctgggagct gtaatggaaa ataaacttca cacaaaagag gaagaaaatc  13440
cccggtctct atgggagcag atgtgggagt gatccattct actggggact atcaggaaga  13500
tttcaaagaa gtgacattga aatgttagca tcaacttgaa aatgaatggt ttactgagat  13560
gtgaaaggac attctgtgca gaaggaaaag ccaggccaag acacatggag tcgagaatag  13620
gctgagatgc gtttagagag ctgactcttg ctaccactgg tcagattggc ttcccttggg  13680
ctctggaaaa tttgtcttta tttatttatt tatttattga gacagagtct tgctctgtca  13740
cccaggctgg agtgcagtgg tgcaaatctt agctcactgc aaccaccacc tcctggtttc  13800
aagtgattct cgtgcctcag cctcctgggt agctgggatt aaaggcacgc accaccacac  13860
ctggctaatt tttttgtatt tttaatagag atggggtttc ttcatgttgg ccaggctgtt  13920
attgaacttg tgacctcaag tgatcccaca gtgttgggat tacaggcgtg agccaccgcg  13980
cccagcctgg gaaattagtc tttaaaagac caagtagaaa aaaagacttt gaacacaatt  14040
ttgaaaggca tttgctactc tgcccatccc cactccctcc aaatttacca tcttaactat  14100
accttaacca aagatgcccc catttttatt ataacctggc tactgtgcat cttttctgga  14160
tttaagtggg gtatttaaac ccgttgacct gcttaattaa gcaaggtgca agtaataaaa  14220
tggaggggaa gataggcaag ccaaaaatgt gttcctgact ggaaggtcac acttctcttg  14280
acgcaggatt atgtagacag tttttgtgag ggcaaaggac gttttgctca tctctatata  14340
atgtcagctg agtatatatt cattgagcac taagtgctca gtgcctacta tgtgccaggc  14400
cctgtgagag ttgctaggac tagatgaatc ggcagtgtct gttggctaat ttagaattga  14460
tcttagatca tcccaggaaa ccttcggttt atttgtctta ccattcaagt gaacaggttt  14520
taatgaaaga tgggtctgtc tgtttttttt cctgtaagga caaccgatgc aagctgtttg  14580
```

-continued

actccccttc cctgtgtagc tccagcactc ggtcagtgtt gaagagacca gaacgatctc 14640
aagaggagtc tccacctgga agtacaaaga ggaggaagag catgtctggg gccagcccca 14700
aagagtcaac taatccagag aaggcccatg aggttagttt cctaggttcc tttttgctct 14760
agcacagata ctgtattttt cagttctaaa aatttctact tagtggttca tttattttct 14820
ttgctaagat ttgctggggt tttgtggggt tgtgtgtgtg tgtgggtgtg tgtgtgtttc 14880
ttttaataga gatgggatct agcgatgttg cccacgctgg tcctgaactc ctagcctcaa 14940
gtgatcctcc tgccttggcc tcccaaagca ctgggattac aagtgtgagc caccacacct 15000
ggctgatact tggctgtttt catgttggtt tttcatttgc tttgagtatg ttcataattg 15060
cctgttgaag catttttaag atggctgctt taaaatcttt gtaagacaaa tccaacatcc 15120
atccatagtg ttgttggcat ctgttgattg tcttttcctg ttcaagttgg gatttttctg 15180
gttcttggta tgaccggtga ttcttccatt gtctcctgta tatttgtgag tctccggtct 15240
atgtaaattt tacgttttag caggcttcct ctgatactgc aagaaaggca tgggtggaag 15300
tccgggttcc ccagcacaaa ctccattgac accttgggtg gggacttgtc tcattattgc 15360
tgggagggag tggaagttca ttccatcccc gtggggtggt gactggggca ctgtatgaca 15420
gctgggcaaa ggtggaggcc cccgcttgct gttggccata attgtttctg tgttgactag 15480
tagaatgatt attgtctaaa agtcttctgc ctcaataggc catcctttgc ctagggagag 15540
caggcttttc ttggggcttg ttgtgttgct gtctccagct tcatgagttg aatggttaac 15600
tcattcgttt ttaattttct tatttctgga taaatctatt tgaatctatg aatttcccac 15660
tgctttagat ttgtctcata acttttgacc tgaaatgttt ttattatcat ttgttgtaga 15720
tattttattt ccttttaatc cagacctatc atggcctatt aaatacttga tttttataaa 15780
ggtctgtctt tttttttttt ttttttgaga cgctctgtcg cccaggctgg agtgcagtgg 15840
cgcgatctca gctcactgca agctctgcct cccgggttca tgccattctc ctgcttcagc 15900
ctcccagtag ctgggactac aggaccccgc caccacgcct ggctaatttt ttgtattttt 15960
agtagagacg gggtttcaca gtgttagcta ggatggtctc catctcctga ccttgtgatc 16020
cgcccgcctc agcctcctaa agtgctggaa ttacaggcgt gagccactgc gcccagccct 16080
gtctgtctaa ttcttcaagt taattcattg cattgctcat agttgtatag gctgttttgt 16140
tgtttcctgt ttctgagaca gggtctctct gtcacccagg ctggagtgca gtggcatgat 16200
ctccgctcac tgcactctcc acctcccagg ctcaagcagt cctcccacct caggctctca 16260
agcagctggg actataggtg tgtgccacca aacccagcta atttttgtat tttttgtaga 16320
gacaggcttt cgccatgttg cgcaggctgg tctcaaactc ctgggctcag ggcaatcctc 16380
ccgccttggc ctcccaaagt gccaggatca taggtgttag acacagcacc tggcatgggt 16440
gagctagtgt catggttgtc tgggcctaag tcctatggtc ctttgtgtta ttttttctat 16500
ttattctgtg gctagacaca caaaacactg gtttatttgt atgtttttcc ttcattatac 16560
tgtccttata ttgaggtttg gtattaagca ttataaaatg gtgaactacc tgtgttttct 16620
gtggcatgaa aaggtttaag taaatctcat cacagtctta aaaggttaga gagatgacag 16680
ctaggaaaac atttagcatt taggcctact gccttatcgt atctgctttt gtttttaatg 16740
ctttatgaat gtttttaaag tattgttcta aaagaatatt ttaataattg catagtattt 16800
ttgttttggg agagttgttt ggttttggtt tttgttcttt ttgtttgtgg ggggcacagg 16860

-continued

gtctagcgct gtcactcagg ctggagtgca ctagcacgat cacagctcag tacagcctcc 16920
atgggctcaa gcactcctcc cgcctcagcc cccctagcag ctgggactac aggcatgcac 16980
caccatgccc agttaattta aaaaaaattt ttttttattt tttgtagaaa agaggtctta 17040
ctgtgttgcc caggctggcc ttgaattcct gggctcaagc agtcctccca cctcagcctc 17100
caaagtatct gggactacag gcacaggcca gtgcacctgg ccgatagtgt tttgacttac 17160
aagtatacta gaaataattg aataaatcac cttctggaca tttagaattt cccttcccct 17220
gattttcctt ttatttctaa ttaaaaatga aattctgggt tataagggca agaacatttg 17280
tgaaacttta cattgtatac atgtcagaaa atatctatca atatttaata atattctggc 17340
tgagtgctgt ggttcactcc tgtaatccca gcactttggg aggacaaggc gggtggatca 17400
cgaggtcagg agttccatac cagcctggcc aatatggtga aaccctgtct ctactgaaaa 17460
tacaaaaaat agctgggtgt ggtggtatga gcctgtaatc ccagctgctc aggaggctga 17520
ggcaggagaa tcgcttgaac ccgggaaatg gaggttgcag tgagcagaga tcacgccact 17580
gcactccagc ctgggtgata gagtgagatt tcatctcaaa aaaaaaaaaa aaagaatatt 17640
cattttcttt ttctttcttt ttgagacacg ctggagtgca gtggcaggat cttggctcac 17700
ggcaacctcc gcctccccag ttcaagcaag tctctgcctc agcctcctga gtagctggga 17760
ttacaggtgc ccactgccac gcctggctaa ttttttttgt atttttagta gaggcggagt 17820
ttcaccaagt tggccaggct ggtcttgaac tcctgacctc aggtgatata tccgccttgg 17880
cctcccaaag tgctggggtt acaggtgtga gccactgtgc ccggccagaa tattcatttt 17940
cttttaccgt aatagagatt gaccttttta tttccatatt tgtaaaactt tttttggata 18000
aaataatact gttaataatt attttgtata tatattggcc ctcatgtttt ggtttatttt 18060
catatatttt aatttttagt attttaaatt ccattgtgga gaaaatgtca agcattgcta 18120
gagtacagtt atcatacctg tgatattgtg aaatatatat ttagtcttct tccccttctg 18180
tcatataact cctaaaatct ttggaatatc caaggtcata tctttttgta tactaatgat 18240
tgatagcttc agggtgggac tggtcactgg aaagacagag acatgattag aggttggaac 18300
tttcagcccc accctccaat gtctacggaa aggagagggg ctaaaggtca agttgatcac 18360
tcatggccaa tggtttaatc aatcatttct atgtaatgaa gcctccctaa aaccttgaaa 18420
ggacagggtt cagagagctt ctggatagct gaacacatgg aggttcctgg aggttggggc 18480
acccaaggag agcatggcag ctccatgtcc cttctcccat acctcaccct atgcatgtct 18540
tcttctatat cctttgaata tccttcataa taaactggta aatgtgtttt cttgagttct 18600
gtgagccact tctagcaaat taaacccaaa gaaggggtca tgggaacccc tacttaaagc 18660
tggtggtcgt agatcagaag atccagaggc ccaactggtg tctgaagggt ggtgtggttt 18720
tgaggactgg gccctcaact tcctgatctg acgctctgca ggtagatagt gtcagaattg 18780
aattggacgg cacccagcta ttttccactg cagaactgct tgcttgcttg cttgcttgct 18840
ggtagggaga aatcccctca tatctggggg taacagcact tctgtcttct gttgctgttg 18900
agtgagggaa taagaaaaat cactttgagt ttgtggggtg ttttcctcac acaaacatat 18960
catacagggc taaatttctg ggtgtttttt tgtttgtttt tttttttgag accaagtctc 19020
gctctgtcgc ccagactgga gtgccatggg tcgatctctg ctcactgcaa cctccgcctc 19080
tcaggttcca gcaattctcc cgcctcagcc tcccaagtag ctgggactac aggcgcccac 19140

-continued

```
caccacgcct ggctaatttt ttatattttt agtagagacg gggtttcacc atgttggcaa  19200
ggctggtctc gaacccctga catcaagtga tccactcacc tcggcctccc aaagtgctgg  19260
gattacaggc atgagccacc acgcccggca gagctaaatt tcttactatc aaatgtcaaa  19320
tatccagtct gggttcagtt ttccccagtt gtctcctaag tacttttaca gtttatttgt  19380
taaaatctgg atccaaataa agtctataca ctgtagttgg tcaataggtc tcttactctt  19440
cagatttcag tgctccatct gtttccctct tgcatatttt tatttgttga agagctcaag  19500
tcatttgtcc tgcaaaggtt cccacagtcc tgtggggtct tcagacattg tcttctatcc  19560
cctatattgc cttaacctaa ctctggaaag gatggattga attccagttc tatttgtttg  19620
gcaagaatat cacataggtg attttgtcta ctttgatcag gaagtataat gtgccaggca  19680
gccattggtg attgctgccc agattttta cttcatttca ccagggtttc agaaagatga  19740
tactgcagtt tttacattct ttcctcatta attagctaga atatatctat aaagagaaac  19800
ttacactcat caaacactgt ggttaccctt gggaaaggca ggatgaatat tggatctctt  19860
tatttgccag tttccaaata atgccctacc aagcatcttc caaagttgaa agcaagactt  19920
atagttgttt ttcaataata atcaggaatt catgggttaa aacatttaat gtgtttctgt  19980
gtattacaag tattatcctt ttactcaaat tatccccttt ttaacagctg gcttcctctt  20040
ctcattgaat aaagctaaga cgtaggccgg gcgcggtggc tcacacctgt aatcccagca  20100
ctttgggagg ccaaggcagg cagatcacct gaggccagga gttcaagacc atctggccaa  20160
catggtgaaa ccccgtctct actaaaaata caaaaattag ctgggcgtgg tggcgcacac  20220
ctatagtctc agctgctctg gaggctgcgg cagaatcgct tgaacctggg aggcagaggt  20280
tacagtgagc caagatcact gtactccagc ctgcactcca ctcctgtact ccactgcact  20340
ccagcctgag cgacagagcg agactctgtc tttttaaaaa aaaaaaaaaa aaaaagttaa  20400
gacatacttt ttctaaactt aatcctagtt ttaacccccc ttgtttcttt ctctttttct  20460
ggagatagtt tcagtctgtc acccaggctg gagtgcagtg gcaccatcac cacttgctgt  20520
agcctcgacc tccccaggct caggtgatcc tcccacttca gcctcccaag tagctgggac  20580
tacaggcaca caccaccatg cccagcttgt gtgtgtgtgt gtgtgtgtgt gtgtgtgtgt  20640
gtgtgtgtgt gtgtgtggac agggtcttgc tctttgatct ggtctggaac tcctgggctc  20700
aagcgattca cctgccttgc ccaggctggt ctggaactcc tgggctcaag tgagtaaccc  20760
accttggcct cccaaagtgc tgggattaca ggcatgagcc aaaccacctg cacccccttt  20820
tttttttttt ttttttaagg aaggaaggaa aacttagttt cagagcaatt cggttagaca  20880
ctggaataat aggagaccta gtcaataacc cacatctgtt tttgttcact ccagattcaa  20940
taaaataaaa taaaaacttc ttactaaatg taggcattaa cattttagtc tcctcgagac  21000
atgctccaag tgaatgtttt cagaagttcc attcagaacc cttgtctcat tctctacctt  21060
tgatttgtta cagactcttc atcagtcttt atccctggca tcttccccca aaggaaccat  21120
tgagaacatt ttggacaatg acccaaggga ccttatagga gacttctcca aggtaattgc  21180
aagcagagct gctctggcaa gtgtaggagg gagtgtgggt atttagaatc ccactcagcc  21240
tgtctccctc cccagggtgg ttcctggcat acctccaaaa ggacacagtt aaaagaatgt  21300
taaaggtagg gaagcaaact tagtttctca tgatcaggta tatgttggtt tctgagactg  21360
tagatatcac tatagctgat gggcagtttt aggtagggag ctgtccacca accaccttga  21420
```

-continued

ttgtaaccca aagactaggc tctctgggaa ctgtgttatt ataaaaatag taattagcag 21480

gatagtgtac agcagaaata aatctgtagc cacactacaa gtctgcatgt tgaaaggtta 21540

tcttagaagg tctgggattg agactgaatt tctccgttca gagaagcttg gccattgagg 21600

gaaaagaccc tccaggaagt ctagaggaag atcttctaat agcctgatat actacatgaa 21660

ggcttggctg cagtaaaata caaggctagg acagggaatg tgtcaatagc agcttcttta 21720

atcacatttt gacttgaagg ttaccagatc aatgttttat tcattaattt agttaacatt 21780

tattgaagca cttatgtgtt aggcagagat tccaaaatga gatacagttc cttccctcaa 21840

agaatttagc atgtggttgg agaggtgaga cgtgaactac atgtaccaca tggtagactg 21900

aaataaattt tagtgaggtg taaacataaa cagcatgccg tgagaagtaa aagggtttat 21960

cttggctagg gggttgggga acgtctgggg ttgggccgtt gatatttcag cccaaatggg 22020

ttttgaagga acggagaatg aagagaacaa gcaaagatcc agctgtatcc tcagttttgg 22080

aaccttctct tgtttggcag ggagagatgt ggtcttacta tgttgctcag gctgtagtgc 22140

agtggctatt cgcacatgct atcatagtac actgtagcct caaacttatg gcctcaagtg 22200

ctccccctgc ctcagcctcc caagtagctg ggactacagg catgcaccac cacactcagt 22260

ggaattttca acttgaattg aggcctggtt atatttgtct taatgggcct atgcatgggg 22320

atagatgaac tcttggctca gccagtcacc ctaatgagta attgctaatg tgtgcatctt 22380

cctcctcaag gctgggctag gtctcttttt ttcctatccc cagtgcctgg caccatgctg 22440

gacatagcag gtgctcagta aatgagtgaa atctgtatgt ttaagtgcta ttcgcagtct 22500

aactactgac gtgtggattc ttgacaaaag caggaggaaa atgagattac ctgaggtttc 22560

tttatttaaa tctgccttac tagctaggta accttggata aggtgctcaa aatggggata 22620

atatacctca ttggtttgtg tgaaaattaa atgtcaggat gttaatgaag taactagaag 22680

agtaactagt gtgtactaac taatgtttgt ttgcttgttt gtttgtttgt tttttgagag 22740

ggagtcttgc tctgtcaccc aggctggagt gcagtggtgc aatctcagct cactgcaacc 22800

tccgcctcct gtgattcaag cgattttccc actccagcct tccgagtagc tgggactata 22860

ggtgcatgct aacgcccggc taatttttgt atttttaaaa gagttggggt ttcaccatgt 22920

tggccaggct tgtctcgaac tcctgacctc aggtgatcca ctagcctcgg cctcccaaag 22980

tgctgggatt acaggtgtga gccaccgtgc ctggccatta atgtttgttt tttaaaagat 23040

gcctcctacc agccagacaa aacagtataa gtgtgaataa tatataggct ttcagattct 23100

ctaagtatct ttttttcttt ttaagggtta tctctttcat acagttgctg ggaaacatca 23160

ggatttaaaa tacatctctc cagaaattgt aagtccatcc ttttgaaacc caccacacat 23220

cgggtacttg aatctagttt tccctgacgg tcaaagttga ttctccctaa ttttctctca 23280

acagatggca tctgttttga atggcaagtt tgccaacctc attaaagagt ttgttatcat 23340

cgactgtcga tacccatatg aatacgaggg aggccacatc aaggtatgga ttcctgagac 23400

ttgctgtaga aggagcccta aacaggatct gtggttttaa agtggggagg acagtgacac 23460

ctggccactt agctcatatg ctagttgtta gaattttgaa acaatacagt gagtgtggaa 23520

ggcatttgat tccgggtgct cttaacgaat gttcccttgt ctgcaatact ctctaccacc 23580

ttgtgctaca gttattcttc atgtttgtcc ctcacaccat cattatcaag gacacttgta 23640

ggaggtaccc actgatgttt aagtcattcg ggggacaaga atgggtttca tagcatttaa 23700

-continued

```
gaggcttggg taccaatgtc tgagcacccc agggctcctg atgaggagaa gaccatctct  23760
ggcaacatgt cctggtgtct gtgccaggct tagtgatgcc tccaaagttg attgttcagc  23820
aagctgcctg gtggaggggc gtgttgggac acacgttccc aagaagaaag gaactgattt  23880
tgccatttta atcttttttt tttttttttt tttgagacag agtccgctct gtcgccaggc  23940
tggagtgcag tggcatgatc tcggatcacc acaacctctg cctcctgggt tcaagcaatt  24000
ctcctgcctc agccttccaa gtagctggga ctacaggtgc acgctgccac gcccggctaa  24060
tttttttttt tttttagtag agacggggtt tcaccatgtt gcccgggctg gtctcaaact  24120
cctgagctca ggcaaaccgc ccgcctcagc ctcccaaagt gctaggatta caggcatgag  24180
ccacggcgcc tggcctgcca ttttaatgtt ttacccgatt aatagtgttt aaatatagta  24240
aggaggtact ttaaatacga tgttactgtt tgccattgtc ttgtggcttt tgaaaatccc  24300
caaatttata aaactaccta taaagtgtac cattgtcata tttgaattgc ccatttccag  24360
tccccatgtg cattactgta tgatgaacgt gtacggaccc acaaagctcc attgagatac  24420
aggtgccaaa tggtctactg ggctattcag ctagaagaac tgcaaaaatc acaaactggt  24480
cttgtggact taaagatcac tcttgatgaa tcattacatc ttgctgaatc attacttcat  24540
gtgtgaaacg tgcaggttcc aagggtctct gttgtcttca cagggtgcag tgaacttgca  24600
catggaagaa gaggttgaag acttcttatt gaagaagccc attgtaccta ctgatggcaa  24660
gcgtgtcatt gttgtgtttc actgcgagtt ttcttctgag agaggtcccc gcatgtgagt  24720
gctgcacgga actgggttct ggggcacagg ctccatgatg cttttgtggg acatggtggt  24780
ttgtggcttg cacttggagc atattttagc atatcaagca acctctggca cataacaagc  24840
cattttcata tgtaatttta tcctgaggtg aactttggct cttccagtct ccccgactgt  24900
ccttagtctg atctgtgggg ctactgttct catgtgacct ccttcaagta caaagtgacc  24960
gttccttatg caaatgccag aaaagtgtga agttctcatt ctgtaaaacc tcatatgata  25020
tgatgctttg aaacacttga tattattacc aatttcaggg tgaaaagaaa aaagggggcc  25080
aaagagctgc ccatcagtca tctgtgtcca ttctaaaagg attgcaagcc tgctgtttgt  25140
caggcactga caataaaaat gtaactacag taaaacacag tacagtaaaa ataccacagc  25200
gagcaggacc aacaaggacc ctactgcagc agagatccaa gtggggatcc gtataaatgc  25260
taggaggaca ggcagagagg taggacagag agtgcagctt aactaggatg gtccaaggga  25320
gacttctgca ggaccacgtt cttatgccac ctttttattt tgaaccaatt tattttaaaa  25380
aacattttta aattaaaaaa tttcaatttt ttttttttga cggagttttg ctcttgttgc  25440
ccaggctgga gtgcagtggc acaatctctt ctcactgcaa cctctgcctc ccaggttcaa  25500
gcagttctcc tgcctcagcc tcccaagtag ctgggattac aggcatgcgc catcacgccc  25560
ggctaatttt gtatttttag tagaggcagg gtttctacat gttggtcagg ctggtcttga  25620
actcccgacc ttaggtgatc cgcctgcctc agcctcccaa agtagtggga ttacaggcgt  25680
gagccactgc acctggcttt ttctttcttt ctttctttct tttttttttt ttttttttgag  25740
gcagaggcac actgttttca cccagactgg aatgcagtgg catgatctcg gctcactgca  25800
acctccacct cctgggttca agcgattctc ctgcctcagc ctcctgagta gctgggataa  25860
caggcgtgca ccaccacacc cggctaattt ttgtatttgt agtagagatg gggtttcac   25920
catgttggcc gggctggtct tgaactcctg acctcaggtg atccacccgc cttgacctcc  25980
```

-continued

caaagtgctg ggattacagg tgtgagccac cacgcccagc agaaatttca aattttgaga 26040
tggggtctta atatattggc caggttggtc tcaaactcct ggcctcaagc aatcctctct 26100
ccttggcctc caaagtgcta ggattgcagg catgagccac tgtgcccacc cctttgaacc 26160
tttttttttt tttgagacgg agtctcgctc tgtcacccag gctggagtgc agtggcacaa 26220
tctcgactta ctgcaagctc cgcctcctgg gttcatgcca ttctcctgcc tcagcctccc 26280
gagtagctgg gactacaggt gcccaccatc acgcctggct aatttttttg tatttttagt 26340
agagacaggg tttcaccatt ttagccagga tggtctccat ctcctgacct tgcgatctgc 26400
ccgtctcggc ctcccaaagt gcttggatta caggcgtgag ccactgcgcc tgacctgaac 26460
cattttaaag ctcaagaaga ggtgaggtta tggctgggca cagtggcacc tgtaatccca 26520
acactttggg aggccgaggc aggaggatca cttgctcggg agtttcagac cagaccaacc 26580
tgggtaacac agtgggacca cacctctaca aaaaatagga ggtgggagga tcgtttgagc 26640
ccagcaggtc gaggctgtgg tgagccatga tcatgccact gtactccagc ctaggtgaca 26700
gagtgagatt ctgtctctct ctcacacaca cacacacaca cacacacaca cacacaaagg 26760
ttatacttgc acacacttca cctgatttta ttaattttta acattttgtc acatttgtat 26820
gtacatgctc tacacacagg caactacagt acaacttgac acttttgact gtcatctcat 26880
aagtccttca aacatctgtc tccaaaggat gctcattcat gaatggaatt attcactcag 26940
cagttttttt gtttgtttgt ttgagatgta gtcttgctct gccacccaga ctggagtgca 27000
gtggtgcgat ctcggatcac tgcaacctcc acctcccggg ttcaagcaat tctcctgcct 27060
caggctccca agtagctggg actacgggtg catgccacca tgccaagcta atgtttgtat 27120
ttttagtaga gacggggttt tgctacattg gccaggctgg tctcgaactc ctgacctcaa 27180
gtgatctacc cgcctcagcc tcccaaagtg ctgggattat aggcctgaaa caaactaatc 27240
tggacacaca tacacacaca tgtaaattgt ttttaaaccc acaaaagatg ctgctctctt 27300
tttttttat ttttgagtca gggtcagtct ctgttgtcca ggctggagta cagtggcagg 27360
agcacagctc actgtagcct ctgcctccca ggctcaagtg attctcccac ctcagcctcc 27420
caggaagcta ggaccacaaa catgcatcac catgcctggc taatttttgt atttttttata 27480
gagacagggt ctcactgtgt tgcccaggct ggtctcaaac tcctgggctc aggcaatcct 27540
ggcctcccaa agtgcttgga ttacaggtgt gagccgccat gcccagccca aaagatgcta 27600
ttctcttaat ctctcatttc cccatcttcc tctttaacac ctctaatata aaggacatta 27660
ttataccata ttgaaacttc acctagatat acagaaaacg ccccatctct gtattctgta 27720
tttgcaagct gcatgtttat atcaccatct acagataccc cttacacttc gttccccact 27780
tgagtttcgc catcactggc tttctgttgt tccactgggg aaagatttgg gattcacagg 27840
tgaactcttt tttttttttt tttttttttt tgagttggaa tctcgccctt tcgcccaggc 27900
tggaatgcag tggcacaatc tcggctcact gcagcctcct cctcccgggt tcaagcattt 27960
ctctgcctca gcctcccaag tagctgggat tacaggcacc tgccaccacg cccagctaat 28020
tttttgtagt tttagtagac acagggtttc actatcttgg ccaggctggt ctcgaactcc 28080
tgacctcgtg atccaccgcc tcagcctccc aaagtgctgg aattacaggt gtgagccacc 28140
gcgcccagcc aaactccttt taaccacagg caaatggttt aagctacgtg ctgcagagga 28200
agctgctgcc catccctcat gagcagctaa caaaggcccc aaaccactga gcttcaagag 28260

-continued

```
aaaatcagca gatactcttg gctcttgtgt accagatacc tttctagata ctgagggaga  28320
agagcagtga acaaaacaga ccaaaaaatc tctgccttca tggaacttac atttcggagt  28380
ggacaggaga tcgagaccag cctggccaac atggtgaaat cccgtctcta ctaaaaatac  28440
aaaaattagc caggcgtggt ggcggacacc tgtaatccca gctactttgg aggctgaggc  28500
aggagcatcc cttgaaccca ggaggcagag gttgcagtga gccgagatcg cgccagtgca  28560
ctccagcctg ggcgacagag tgagactcca tctcaaaaaa aaaaaaaaaa aggtgactgg  28620
gtgcggtggc taactcctgt aatcccaaca ctttgggagg ccaaggcagg tggatcacaa  28680
ggtcaagagg tcgagaccat cctggccaac atggtgaagc cccatctcta ctaaacatac  28740
aaaaatcagc tgggcggtgg cacgcgcctg tagtcccagc tatttgggag gctgaggcag  28800
gagaattgct tgaacccggg agacggagat tgcagtgagc ctgaggtcac gcccttgcac  28860
tccagcctgg tgacagagtg agattctgtc tcagaaaaaa gaaggtgctt cagaggaaaa  28920
taaggccagt ggtggagctc ccaggcaggc tggaggaaca gtagtggggc tgcaggggct  28980
ggcgtggggt gaacaagggg cagagcagca gtaggggcct gtagagctgt aggcctctga  29040
aggacttggg gcctcgggct ttttcaccaa gggcgaggca gtaagcagcc attggctgga  29100
tattgaagac cattcagtcc aagtagagga tagaaacagg gccccgaggc aggagcaagt  29160
tcagcctctt taaaagccac agaaagaaac aagcccactt atgtagaagg aggtgagtga  29220
gggagcctgg ctgaagagaa ggctggggtg gagaaagcaa ggggaagctg gctcatatag  29280
tgaaaacttg ccacaaactg tggtggtgaa ggtgctgggc aggactcaga tgctaggatt  29340
gttggagaaa ggaaaattgg ggaagagacc atctactggc actggctgtg agtgctgctc  29400
atgtggccag gaacttgcaa ctcagcactg attgtttcta agaataaatg tcaagttggg  29460
aagatgtgta taagggggac ttagaccaca actgctgctt tgactgcgtt gccttgttgc  29520
tgtgctggaa aagctaaccc tgctttggcc ttccttcccc tgactaggtg ccggtatgtg  29580
agagagagag atcgcctggg taatgaatac cccaaactcc actaccctga gctgtatgtc  29640
ctgaaggggg gatacaagga gttctttatg aaatgccagg taagactggg gttgtggaga  29700
gcatctctcc tcccctgccc ccagtggtag actaatggat ctgtctggtg gtcatgactt  29760
tctttccagg ggtcggggga caaggtgggt atctgctgaa cccaaagaaa gcccctgtag  29820
aactggctcc ctaggtctgc ctggccgctt tcagccttgt agccctaggc agagaggaaa  29880
ccaggttgtg ggtgtgaggc aggtgtaccc taaccttatt ctctcctgtc ccctcagtct  29940
tactgtgagc cccctagcta ccggcccatg caccacgagg actttaaaga agacctgaag  30000
aagttccgca ccaagagccg gacctgggca ggggagaaga gcaagaggga gatgtacagt  30060
cgtctgaaga agctctgagg gcggcaggac cagccagcag cagcccaagc ttccctccat  30120
ccccctttac cctctttgct gcagagaaac ttaagcaaag gggacagctg tgtgacattt  30180
ggagaggggg cctgggactt ccatgcctta aacctacctc ccacactccc aaggttggag  30240
cccagggcat cttgctggct acgcctcttc tgtccctgtt agacgtcctc cgtccatatc  30300
agaactgtgc cacaatgcag ttctgagcac cgtgtcaagc tgctctgagc cacagtggga  30360
tgaaccagcc ggggccttat cgggctccag ccatctcatg aggggagagg agacggaggg  30420
gagtagagaa gttacacaga aatgctgctg gccaaatagc aaagacaacc tgggaaggaa  30480
aggtctttgt gggataatcc atatgtttaa tttattcaac ttcatcaatc actttatttt  30540
```

-continued

```
atttttttt ctaactcctg gagacttatt ttactgcttc attaggttga aatactgcca  30600

ttctaggtag ggttttatta tcccagggac tacctcggct tttaatttaa aaaaaaaaaa  30660

gaagtgggta agaaaatgca aacctgttat aagttatcgg acagaaagct aggtgctctg  30720

tcacccccag gaggcgctgt ggtactgggg ctgctgctat ttaagccaag aactgaggtc  30780

ctggtgagag cgttggaccc aggcttggct gcctgacata agctaaatct cccagaccca  30840

ccactggcta ccgatatcta tttggtggga ggtgtggccc tgttcttcct caccccagtt  30900

ccatgacatt ggctggtata ggagccacag tcaggaaagc acttgaggca gcatctgttg  30960

ggccaccccc ggctcagtgc tggaatgttg cagtgtaggt ttcccaggga agggggggtgg  31020

gggtaggtgg gctccacagg atgggggagg agcatgtcca ctgagtatct tccttatgtt  31080

gctgtgatat tgatagcttt tattttctaa tttttaaaaa atggtcatat tatgagtcaa  31140

agagtatcaa atcagtgttg gatggaccac ccaagggtga ggagaggggc tggaagccct  31200

gggcattagg agaagggagt gggtgctggc atggacatga ctggatagaa ttttctcagg  31260

agggagcttg gtggattttg aaggtaaaac tttctgggtt tatcatgttt taattttaga  31320

gacagggagt gatgaatcat caccggttgt ccccttatct aactccataa aagtgggaat  31380

ttcaaaagaa cacctcatcc aaggagctgg ggcagacttc attgattcta gagagacctg  31440

tttcagtgcc tactcatccc tgccctctgg tgccagcctc cttaccatca cggcttcact  31500

gaggtgtagg tgggtttttc ttaaacagga gacagtctct cccctcttac ctcaacttct  31560

tggggtggga atcagtgata ctggagatgg ctagttgctg tgttacgggt ttgagttaca  31620

tttggctata aaacaatctt gttgggaaaa atgtggggga gaggacttct tcctacacgc  31680

gcattgagac agattccaac tggttaatga tattgtttgt aagaaagaga ttctgttggt  31740

tgactgccta aagagaaagg tgggatggcc ttcagattat accagcttag ctagcattac  31800

taaccaactg ttggaagctc tgaaaataaa agatcttgaa cccatgctct ctgcctagtt  31860

cttgatgg                                                           31868
```

<210> SEQ ID NO 809
<211> LENGTH: 524
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 809

```
Met Glu Leu Gly Pro Glu Pro Pro His Arg Arg Arg Leu Leu Phe Ala
1               5                   10                  15

Cys Ser Pro Pro Pro Ala Ser Gln Pro Val Val Lys Ala Leu Phe Gly
            20                  25                  30

Ala Ser Ala Ala Gly Gly Leu Ser Pro Val Thr Asn Leu Thr Val Thr
        35                  40                  45

Met Asp Gln Leu Gln Gly Leu Gly Ser Asp Tyr Glu Gln Pro Leu Glu
    50                  55                  60

Val Lys Asn Asn Ser Asn Leu Gln Arg Met Gly Ser Ser Glu Ser Thr
65                  70                  75                  80

Asp Ser Gly Phe Cys Leu Asp Ser Pro Gly Pro Leu Asp Ser Lys Glu
                85                  90                  95

Asn Leu Glu Asn Pro Met Arg Arg Ile His Ser Leu Pro Gln Lys Leu
            100                 105                 110

Leu Gly Cys Ser Pro Ala Leu Lys Arg Ser His Ser Asp Ser Leu Asp
```

-continued

```
        115                 120                 125

His Asp Ile Phe Gln Leu Ile Asp Pro Asp Glu Asn Lys Glu Asn Glu
    130                 135                 140

Ala Phe Glu Phe Lys Lys Pro Val Arg Pro Val Ser Arg Gly Cys Leu
145                 150                 155                 160

His Ser His Gly Leu Gln Glu Gly Lys Asp Leu Phe Thr Gln Arg Gln
                165                 170                 175

Asn Ser Ala Pro Ala Arg Met Leu Ser Ser Asn Glu Arg Asp Ser Ser
            180                 185                 190

Glu Pro Gly Asn Phe Ile Pro Leu Phe Thr Pro Gln Ser Pro Val Thr
        195                 200                 205

Ala Thr Leu Ser Asp Glu Asp Asp Gly Phe Val Asp Leu Leu Asp Gly
    210                 215                 220

Glu Asn Leu Lys Asn Glu Glu Glu Thr Pro Ser Cys Met Ala Ser Leu
225                 230                 235                 240

Trp Thr Ala Pro Leu Val Met Arg Thr Thr Asn Leu Asp Asn Arg Cys
                245                 250                 255

Lys Leu Phe Asp Ser Pro Ser Leu Cys Ser Ser Ser Thr Arg Ser Val
            260                 265                 270

Leu Lys Arg Pro Glu Arg Ser Gln Glu Glu Ser Pro Pro Gly Ser Thr
        275                 280                 285

Lys Arg Arg Lys Ser Met Ser Gly Ala Ser Pro Lys Glu Ser Thr Asn
    290                 295                 300

Pro Glu Lys Ala His Glu Thr Leu His Gln Ser Leu Ser Leu Ala Ser
305                 310                 315                 320

Ser Pro Lys Gly Thr Ile Glu Asn Ile Leu Asp Asn Asp Pro Arg Asp
                325                 330                 335

Leu Ile Gly Asp Phe Ser Lys Gly Tyr Leu Phe His Thr Val Ala Gly
            340                 345                 350

Lys His Gln Asp Leu Lys Tyr Ile Ser Pro Glu Ile Met Ala Ser Val
        355                 360                 365

Leu Asn Gly Lys Phe Ala Asn Leu Ile Lys Glu Phe Val Ile Ile Asp
    370                 375                 380

Cys Arg Tyr Pro Tyr Glu Tyr Glu Gly Gly His Ile Lys Gly Ala Val
385                 390                 395                 400

Asn Leu His Met Glu Glu Glu Val Glu Asp Phe Leu Leu Lys Lys Pro
                405                 410                 415

Ile Val Pro Thr Asp Gly Lys Arg Val Ile Val Val Phe His Cys Glu
            420                 425                 430

Phe Ser Ser Glu Arg Gly Pro Arg Met Cys Arg Tyr Val Arg Glu Arg
        435                 440                 445

Asp Arg Leu Gly Asn Glu Tyr Pro Lys Leu His Tyr Pro Glu Leu Tyr
    450                 455                 460

Val Leu Lys Gly Gly Tyr Lys Glu Phe Phe Met Lys Cys Gln Ser Tyr
465                 470                 475                 480

Cys Glu Pro Pro Ser Tyr Arg Pro Met His His Glu Asp Phe Lys Glu
                485                 490                 495

Asp Leu Lys Lys Phe Arg Thr Lys Ser Arg Thr Trp Ala Gly Glu Lys
            500                 505                 510

Ser Lys Arg Glu Met Tyr Ser Arg Leu Lys Lys Leu
        515                 520
```

-continued

<210> SEQ ID NO 810
<211> LENGTH: 2940
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 810

gccagctgtg ccggcgtttg ttggctgccc tgcgcccggc cctccagcca gccttctgcc 60

ggccccgccg cgatggaggt gccccagccg gagcccgcgc caggctcggc tctcagtcca 120

gcaggcgtgt gcggtggcgc ccagcgtccg ggccacctcc cgggcctcct gctgggatct 180

catggcctcc tggggtcccc ggtgcgggcg gccgcttcct cgccggtcac caccctcacc 240

cagaccatgc acgacctcgc cgggctcggc agccgcagcc gcctgacgca cctatccctg 300

tctcgacggg catccgaatc ctccctgtcg tctgaatcct ccgaatcttc tgatgcaggt 360

ctctgcatgg attcccccag ccctatggac ccccacatgg cggagcagac gtttgaacag 420

gccatccagg cagccagccg gatcattcga aacgagcagt ttgccatcag acgcttccag 480

tctatgccgg tgaggctgct gggccacagc cccgtgcttc ggaacatcac caactcccag 540

gcgcccgacg gccggaggaa gagcgaggcg ggcagtggag ctgccagcag ctctggggaa 600

gacaaggaga atgatggatt tgtcttcaag atgccatgga agcccacaca tcccagctcc 660

acccatgctc tggcagagtg ggccagccgc agggaagcct ttgcccagag acccagctcg 720

gcccccgacc tgatgtgtct cagtcctgac cggaagatgg aagtggagga gctcagcccc 780

ctggccctag gtcgcttctc tctgacccct gcagaggggg atactgagga agatgatgga 840

tttgtggaca tcctagagag tgacttaaag gatgatgatg cagttccccc aggcatggag 900

agtctcatta gtgccccact ggtcaagacc ttggaaaagg aagaggaaaa ggacctcgtc 960

atgtacagca agtgccagcg gctcttccgc tctccgtcca tgccctgcag cgtgatccgg 1020

cccatcctca agaggctgga gcggccccag gacagggaca cgcccgtgca gaataagcgg 1080

aggcggagcg tgacccctcc tgaggagcag caggaggctg aggaacctaa agcccgcgtc 1140

ctccgctcaa aatcactgtg tcacgatgag atcgagaacc tcctggacag tgaccaccga 1200

gagctgattg gagattactc taaggccttc ctcctacaga cagtagacgg aaagcaccaa 1260

gacctcaagt acatctcacc agaaacgatg gtggccctat tgacgggcaa gttcagcaac 1320

atcgtggata agtttgtgat tgtagactgc agataccccct atgaatatga aggcgggcac 1380

atcaagactg cggtgaactt gcccctggaa cgcgacgccg agagcttcct actgaagagc 1440

cccatcgcgc cctgtagcct ggacaagaga gtcatcctca ttttccactg tgaattctca 1500

tctgagcgtg ggccccgcat gtgccgtttc atcagggaac gagaccgtgc tgtcaacgac 1560

taccccagcc tctactaccc tgagatgtat atcctgaaag gcggctacaa ggagttcttc 1620

cctcagcacc cgaacttctg tgaaccccag gactaccggc ccatgaacca cgaggccttc 1680

aaggatgagc taaagacctt ccgcctcaag actcgcagct gggctgggga gcggagccgg 1740

cgggagctct gtagccggct gcaggaccag tgaggggcct gcgccagtcc tgctacctcc 1800

cttgcctttc gaggcctgaa gccagctgcc ctatgggcct gccgggctga gggcctgctg 1860

gaggcctcag gtgctgtcca tgggaaagat ggtgtggtgt cctgcctgtc tgccccagcc 1920

cagattcccc tgtgtcatcc catcattttc catatcctgg tgccccccac ccctggaaga 1980

gcccagtctg ttgagttagt taagttgggt taataccagc ttaaaggcag tattttgtgt 2040

-continued

```
cctccaggag cttcttgttt ccttgttagg gttaaccctt catcttcctg tgtcctgaaa   2100

cgctcctttg tgtgtgtgtc agctgaggct ggggagagcc gtggtccctg aggatgggtc   2160

agagctaaac tccttcctgg cctgagagtc agctctctgc cctgtgtact tcccgggcca   2220

gggctgcccc taatctctgt aggaaccgtg gtatgtctgc catgttgccc ctttctcttt   2280

tcccctttcc tgtcccacca tacgagcacc tccagcctga acagaagctc ttactctttc   2340

ctatttcagt gttacctgtg tgcttggtct gtttgacttt acgcccatct caggacactt   2400

ccgtagactg tttaggttcc cctgtcaaat atcagttacc cactcggtcc cagttttgtt   2460

gccccagaaa gggatgttat tatccttggg ggctcccagg gcaagggtta aggcctgaat   2520

catgagcctg ctggaagccc agcccctact gctgtgaacc ctggggcctg actgctcaga   2580

acttgctgct gtcttgttgc ggatggatgg aaggttggat ggatgggtgg atggccgtgg   2640

atggccgtgg atgcgcagtg ccttgcatac ccaaaccagg tgggagcgtt ttgttgagca   2700

tgacacctgc agcaggaata tatgtgtgcc tatttgtgtg gacaaaaata tttacactta   2760

gggtttggag ctattcaaga ggaaatgtca cagaagcagc taaaccaagg actgagcacc   2820

ctctggattc tgaatctcaa gatgggggca gggctgtgct tgaaggccct gctgagtcat   2880

ctgttagggc cttggttcaa taaagcactg agcaagttga gaaaaaaaaa aaaaaaaaaa   2940
```

```
<210> SEQ ID NO 811
<211> LENGTH: 566
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 811

Met Glu Val Pro Gln Pro Glu Pro Ala Pro Gly Ser Ala Leu Ser Pro
1               5                   10                  15

Ala Gly Val Cys Gly Gly Ala Gln Arg Pro Gly His Leu Pro Gly Leu
            20                  25                  30

Leu Leu Gly Ser His Gly Leu Leu Gly Ser Pro Val Arg Ala Ala Ala
        35                  40                  45

Ser Ser Pro Val Thr Thr Leu Thr Gln Thr Met His Asp Leu Ala Gly
    50                  55                  60

Leu Gly Ser Arg Ser Arg Leu Thr His Leu Ser Leu Ser Arg Arg Ala
65                  70                  75                  80

Ser Glu Ser Ser Leu Ser Ser Glu Ser Ser Glu Ser Ser Asp Ala Gly
                85                  90                  95

Leu Cys Met Asp Ser Pro Ser Pro Met Asp Pro His Met Ala Glu Gln
            100                 105                 110

Thr Phe Glu Gln Ala Ile Gln Ala Ala Ser Arg Ile Ile Arg Asn Glu
        115                 120                 125

Gln Phe Ala Ile Arg Arg Phe Gln Ser Met Pro Val Arg Leu Leu Gly
    130                 135                 140

His Ser Pro Val Leu Arg Asn Ile Thr Asn Ser Gln Ala Pro Asp Gly
145                 150                 155                 160

Arg Arg Lys Ser Glu Ala Gly Ser Gly Ala Ala Ser Ser Ser Gly Glu
                165                 170                 175

Asp Lys Glu Asn Asp Gly Phe Val Phe Lys Met Pro Trp Lys Pro Thr
            180                 185                 190

His Pro Ser Ser Thr His Ala Leu Ala Glu Trp Ala Ser Arg Arg Glu
```

-continued

```
        195                 200                 205

Ala Phe Ala Gln Arg Pro Ser Ser Ala Pro Asp Leu Met Cys Leu Ser
    210                 215                 220

Pro Asp Arg Lys Met Glu Val Glu Glu Leu Ser Pro Leu Ala Leu Gly
225                 230                 235                 240

Arg Phe Ser Leu Thr Pro Ala Glu Gly Asp Thr Glu Glu Asp Asp Gly
                245                 250                 255

Phe Val Asp Ile Leu Glu Ser Asp Leu Lys Asp Asp Asp Ala Val Pro
            260                 265                 270

Pro Gly Met Glu Ser Leu Ile Ser Ala Pro Leu Val Lys Thr Leu Glu
        275                 280                 285

Lys Glu Glu Glu Lys Asp Leu Val Met Tyr Ser Lys Cys Gln Arg Leu
    290                 295                 300

Phe Arg Ser Pro Ser Met Pro Cys Ser Val Ile Arg Pro Ile Leu Lys
305                 310                 315                 320

Arg Leu Glu Arg Pro Gln Asp Arg Asp Thr Pro Val Gln Asn Lys Arg
                325                 330                 335

Arg Arg Ser Val Thr Pro Pro Glu Glu Gln Gln Glu Ala Glu Glu Pro
            340                 345                 350

Lys Ala Arg Val Leu Arg Ser Lys Ser Leu Cys His Asp Glu Ile Glu
        355                 360                 365

Asn Leu Leu Asp Ser Asp His Arg Glu Leu Ile Gly Asp Tyr Ser Lys
    370                 375                 380

Ala Phe Leu Leu Gln Thr Val Asp Gly Lys His Gln Asp Leu Lys Tyr
385                 390                 395                 400

Ile Ser Pro Glu Thr Met Val Ala Leu Leu Thr Gly Lys Phe Ser Asn
                405                 410                 415

Ile Val Asp Lys Phe Val Ile Val Asp Cys Arg Tyr Pro Tyr Glu Tyr
            420                 425                 430

Glu Gly Gly His Ile Lys Thr Ala Val Asn Leu Pro Leu Glu Arg Asp
        435                 440                 445

Ala Glu Ser Phe Leu Leu Lys Ser Pro Ile Ala Pro Cys Ser Leu Asp
    450                 455                 460

Lys Arg Val Ile Leu Ile Phe His Cys Glu Phe Ser Ser Glu Arg Gly
465                 470                 475                 480

Pro Arg Met Cys Arg Phe Ile Arg Glu Arg Asp Arg Ala Val Asn Asp
                485                 490                 495

Tyr Pro Ser Leu Tyr Tyr Pro Glu Met Tyr Ile Leu Lys Gly Gly Tyr
            500                 505                 510

Lys Glu Phe Phe Pro Gln His Pro Asn Phe Cys Glu Pro Gln Asp Tyr
        515                 520                 525

Arg Pro Met Asn His Glu Ala Phe Lys Asp Glu Leu Lys Thr Phe Arg
    530                 535                 540

Leu Lys Thr Arg Ser Trp Ala Gly Glu Arg Ser Arg Arg Glu Leu Cys
545                 550                 555                 560

Ser Arg Leu Gln Asp Gln
                565
```

<210> SEQ ID NO 812
<211> LENGTH: 2115
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

-continued

<400> SEQUENCE: 812

```
ggtcaacgcc tgcggctgtt gatattcttg ctcagaggcc gtaactttgg ccttctgctc     60
agggaagact ctgagtccga cgttggccta cccagtcgga aggcagagct gcaatctagt    120
taactacctc ctttccccta gatttccttt cattctgctc aagtcttcgc ctgtgtccga    180
tccctatcta ctttctctcc tcttgtagca agcctcagac tccaggcttg agctaggttt    240
tgtttttctc ctggtgagaa ttcgaagacc atgtctacgg aactcttctc atccacaaga    300
gaggaaggaa gctctggctc aggacccagt tttaggtcta atcaaaggaa aatgttaaac    360
ctgctcctgg agagagacac ttcctttacc gtctgtccag atgtccctag aactccagtg    420
ggcaaatttc ttggtgattc tgcaaaccta agcattttgt ctggaggaac cccaaaatgt    480
tgcctcgatc tttcgaatct tagcagtggg gagataactg ccactcagct taccacttct    540
gcagaccttg atgaaactgg tcacctggat tcttcaggac ttcaggaagt gcatttagct    600
gggatgaatc atgaccagca cctaatgaaa tgtagcccag cacagcttct ttgtagcact    660
ccgaatggtt tggaccgtgg ccatagaaag agagatgcaa tgtgtagttc atctgcaaat    720
aaagaaaatg acaatggaaa cttggtggac agtgaaatga aatatttggg cagtcccatt    780
actactgttc caaaattgga taaaaatcca aacctaggag aagaccaggc agaagagatt    840
tcagatgaat taatggagtt ttccctgaaa gatcaagaag caaaggtgag cagaagtggc    900
ctatatcgct ccccgtcgat gccagagaac ttgaacaggc caagactgaa gcaggtggaa    960
aaattcaagg acaacacaat accagataaa gttaaaaaaa agtatttttc tggccaagga   1020
aagctcagga agggcttatg tttaaagaag acagtctctc tgtgtgacat tactatcact   1080
cagatgctgg aggaagattc taaccagggg cacctgattg gtgatttttc caaggtatgt   1140
gcgctgccaa ccgtgtcagg gaaacaccaa gatctgaagt atgtcaaccc agaaacagtg   1200
gctgccttac tgtcggggaa gttccagggt ctgattgaga agttttatgt cattgattgt   1260
cgctatccat atgagtatct gggaggacac atccagggag ccttaaactt atatagtcag   1320
gaagaactgt ttaacttctt tctgaagaag cccatcgtcc ctttggacac ccagaagaga   1380
ataatcatcg tgttccactg tgaattctcc tcagagaggg gcccccgaat gtgccgctgt   1440
ctgcgtgaag aggacaggtc tctgaaccag tatcctgcat tgtactaccc agagctatat   1500
atccttaaag gcggctacag agacttcttt ccagaatata tggaactgtg tgaaccacag   1560
agctactgcc ctatgcatca tcaggaccac aagactgagt tgctgaggtg tcgaagccag   1620
agcaaagtgc aggaaggggа gcggcagctg cgggagcaga ttgcccttct ggtgaaggac   1680
atgagcccat gataacattc cagccactgg ctgctaacaa gtcaccaaaa agacactgca   1740
gaaaccctga gcagaaagag gccttctgga tggccaaacc caagattatt aaaagatgtc   1800
tctgcaaacc aacaggctac caacttgtat ccaggcctgg gaatggatta ggtttcagca   1860
gagctgaaag ctggtggcag agtcctggag ctggctctat aaggcagcct tgagttgcat   1920
agagatttgt attggttcag ggaactctgg cattcctttt cccaactcct catgtcttct   1980
cacaagccag ccaactcttt ctctctgggc ttcgggctat gcaagagcgt tgtctacctt   2040
ctttctttgt attttccttc tttgtttccc cctctttctt ttttaaaaat ggaaaaataa   2100
acactacaga atgag                                                    2115
```

-continued

<210> SEQ ID NO 813
<211> LENGTH: 473
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 813

```
Met Ser Thr Glu Leu Phe Ser Ser Thr Arg Glu Glu Gly Ser Ser Gly
1               5                   10                  15

Ser Gly Pro Ser Phe Arg Ser Asn Gln Arg Lys Met Leu Asn Leu Leu
            20                  25                  30

Leu Glu Arg Asp Thr Ser Phe Thr Val Cys Pro Asp Val Pro Arg Thr
        35                  40                  45

Pro Val Gly Lys Phe Leu Gly Asp Ser Ala Asn Leu Ser Ile Leu Ser
    50                  55                  60

Gly Gly Thr Pro Lys Cys Cys Leu Asp Leu Ser Asn Leu Ser Ser Gly
65                  70                  75                  80

Glu Ile Thr Ala Thr Gln Leu Thr Thr Ser Ala Asp Leu Asp Glu Thr
                85                  90                  95

Gly His Leu Asp Ser Ser Gly Leu Gln Glu Val His Leu Ala Gly Met
            100                 105                 110

Asn His Asp Gln His Leu Met Lys Cys Ser Pro Ala Gln Leu Leu Cys
        115                 120                 125

Ser Thr Pro Asn Gly Leu Asp Arg Gly His Arg Lys Arg Asp Ala Met
    130                 135                 140

Cys Ser Ser Ser Ala Asn Lys Glu Asn Asp Asn Gly Asn Leu Val Asp
145                 150                 155                 160

Ser Glu Met Lys Tyr Leu Gly Ser Pro Ile Thr Thr Val Pro Lys Leu
                165                 170                 175

Asp Lys Asn Pro Asn Leu Gly Glu Asp Gln Ala Glu Glu Ile Ser Asp
            180                 185                 190

Glu Leu Met Glu Phe Ser Leu Lys Asp Gln Glu Ala Lys Val Ser Arg
        195                 200                 205

Ser Gly Leu Tyr Arg Ser Pro Ser Met Pro Glu Asn Leu Asn Arg Pro
    210                 215                 220

Arg Leu Lys Gln Val Glu Lys Phe Lys Asp Asn Thr Ile Pro Asp Lys
225                 230                 235                 240

Val Lys Lys Lys Tyr Phe Ser Gly Gln Gly Lys Leu Arg Lys Gly Leu
                245                 250                 255

Cys Leu Lys Lys Thr Val Ser Leu Cys Asp Ile Thr Ile Thr Gln Met
            260                 265                 270

Leu Glu Glu Asp Ser Asn Gln Gly His Leu Ile Gly Asp Phe Ser Lys
        275                 280                 285

Val Cys Ala Leu Pro Thr Val Ser Gly Lys His Gln Asp Leu Lys Tyr
    290                 295                 300

Val Asn Pro Glu Thr Val Ala Ala Leu Leu Ser Gly Lys Phe Gln Gly
305                 310                 315                 320

Leu Ile Glu Lys Phe Tyr Val Ile Asp Cys Arg Tyr Pro Tyr Glu Tyr
                325                 330                 335

Leu Gly Gly His Ile Gln Gly Ala Leu Asn Leu Tyr Ser Gln Glu Glu
            340                 345                 350

Leu Phe Asn Phe Phe Leu Lys Lys Pro Ile Val Pro Leu Asp Thr Gln
        355                 360                 365
```

-continued

Lys Arg Ile Ile Ile Val Phe His Cys Glu Phe Ser Ser Glu Arg Gly
370 375 380

Pro Arg Met Cys Arg Cys Leu Arg Glu Glu Asp Arg Ser Leu Asn Gln
385 390 395 400

Tyr Pro Ala Leu Tyr Tyr Pro Glu Leu Tyr Ile Leu Lys Gly Gly Tyr
405 410 415

Arg Asp Phe Phe Pro Glu Tyr Met Glu Leu Cys Glu Pro Gln Ser Tyr
420 425 430

Cys Pro Met His His Gln Asp His Lys Thr Glu Leu Leu Arg Cys Arg
435 440 445

Ser Gln Ser Lys Val Gln Glu Gly Glu Arg Gln Leu Arg Glu Gln Ile
450 455 460

Ala Leu Leu Val Lys Asp Met Ser Pro
465 470

<210> SEQ ID NO 814
<211> LENGTH: 1896
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 814

ggtcaacgcc tgcggctgtt gatattcttg ctcagaggcc gtaactttgg ccttctgctc 60

agggaagact ctgagtccga cgttggccta cccagtcgga aggcagagct gcaatctagt 120

taactacctc ctttccccta gatttccttt cattctgctc aagtcttcgc ctgtgtccga 180

tccctatcta ctttctctcc tcttgtagca agcctcagac tccaggcttg agctaggttt 240

tgtttttctc ctggtgagaa ttcgaagacc atgtctacgg aactcttctc atccacaaga 300

gaggaaggaa gctctggctc aggacccagt tttaggtcta atcaaaggaa aatgttaaac 360

ctgctcctgg agagagacac ttcctttacc gtctgtccag atgtccctag aactccagtg 420

ggcaaatttc ttggtgattc tgcaaaccta agcattttgt ctgggtcacc tggattcttc 480

aggacttcag gaagtgcatt tagctgggat gacaatggaa acttggtgga cagtgaaatg 540

aaatatttgg gcagtcccat tactactgtt ccaaaattgg ataaaaatcc aaacctagga 600

gaagaccagg cagaagagat ttcagatgaa ttaatggagt tttccctgaa agatcaagaa 660

gcaaaggtga gcagaagtgg cctatatcgc tccccgtcga tgccagagaa cttgaacagg 720

ccaagactga agcaggtgga aaaattcaag gacaacacaa taccagataa agttaaaaaa 780

aagtattttt ctggccaagg aaagctcagg aagggcttat gtttaaagaa gacagtctct 840

ctgtgtgaca ttactatcac tcagatgctg gaggaagatt ctaaccaggg gcacctgatt 900

ggtgattttt ccaaggtatg tgcgctgcca accgtgtcag ggaaacacca agatctgaag 960

tatgtcaacc cagaaacagt ggctgcctta ctgtcgggga agttccaggg tctgattgag 1020

aagttttatg tcattgattg tcgctatcca tatgagtatc tgggaggaca catccaggga 1080

gccttaaact tatatagtca ggaagaactg tttaacttct ttctgaagaa gcccatcgtc 1140

cctttggaca cccagaagag aataatcatc gtgttccact gtgaattctc ctcagagagg 1200

ggccccccgaa tgtgccgctg tctgcgtgaa gaggacaggt ctctgaacca gtatcctgca 1260

ttgtactacc cagagctata tatccttaaa ggcggctaca gagacttctt tccagaatat 1320

atggaactgt gtgaaccaca gagctactgc cctatgcatc atcaggacca caagactgag 1380

ttgctgaggt gtcgaagcca gagcaaagtg caggaagggg agcggcagct gcgggagcag 1440

-continued

```
attgcccttc tggtgaagga catgagccca tgataacatt ccagccactg gctgctaaca   1500

agtcaccaaa aagacactgc agaaaccctg agcagaaaga ggccttctgg atggccaaac   1560

ccaagattat taaaagatgt ctctgcaaac caacaggcta ccaacttgta tccaggcctg   1620

ggaatggatt aggtttcagc agagctgaaa gctggtggca gagtcctgga gctggctcta   1680

taaggcagcc ttgagttgca tagagatttg tattggttca gggaactctg gcattccttt   1740

tcccaactcc tcatgtcttc tcacaagcca gccaactctt tctctctggg cttcgggcta   1800

tgcaagagcg ttgtctacct tctttctttg tattttcctt ctttgtttcc ccctctttct   1860

tttttaaaaa tggaaaaata aacactacag aatgag                             1896


<210> SEQ ID NO 815
<211> LENGTH: 400
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 815

Met Ser Thr Glu Leu Phe Ser Ser Thr Arg Glu Glu Gly Ser Ser Gly
1               5                   10                  15

Ser Gly Pro Ser Phe Arg Ser Asn Gln Arg Lys Met Leu Asn Leu Leu
            20                  25                  30

Leu Glu Arg Asp Thr Ser Phe Thr Val Cys Pro Asp Val Pro Arg Thr
        35                  40                  45

Pro Val Gly Lys Phe Leu Gly Asp Ser Ala Asn Leu Ser Ile Leu Ser
    50                  55                  60

Gly Ser Pro Gly Phe Phe Arg Thr Ser Gly Ser Ala Phe Ser Trp Asp
65                  70                  75                  80

Asp Asn Gly Asn Leu Val Asp Ser Glu Met Lys Tyr Leu Gly Ser Pro
                85                  90                  95

Ile Thr Thr Val Pro Lys Leu Asp Lys Asn Pro Asn Leu Gly Glu Asp
            100                 105                 110

Gln Ala Glu Glu Ile Ser Asp Glu Leu Met Glu Phe Ser Leu Lys Asp
        115                 120                 125

Gln Glu Ala Lys Val Ser Arg Ser Gly Leu Tyr Arg Ser Pro Ser Met
    130                 135                 140

Pro Glu Asn Leu Asn Arg Pro Arg Leu Lys Gln Val Glu Lys Phe Lys
145                 150                 155                 160

Asp Asn Thr Ile Pro Asp Lys Val Lys Lys Lys Tyr Phe Ser Gly Gln
                165                 170                 175

Gly Lys Leu Arg Lys Gly Leu Cys Leu Lys Lys Thr Val Ser Leu Cys
            180                 185                 190

Asp Ile Thr Ile Thr Gln Met Leu Glu Glu Asp Ser Asn Gln Gly His
        195                 200                 205

Leu Ile Gly Asp Phe Ser Lys Val Cys Ala Leu Pro Thr Val Ser Gly
    210                 215                 220

Lys His Gln Asp Leu Lys Tyr Val Asn Pro Glu Thr Val Ala Ala Leu
225                 230                 235                 240

Leu Ser Gly Lys Phe Gln Gly Leu Ile Glu Lys Phe Tyr Val Ile Asp
                245                 250                 255

Cys Arg Tyr Pro Tyr Glu Tyr Leu Gly Gly His Ile Gln Gly Ala Leu
            260                 265                 270
```

-continued

```
Asn Leu Tyr Ser Gln Glu Glu Leu Phe Asn Phe Phe Leu Lys Lys Pro
        275                 280                 285

Ile Val Pro Leu Asp Thr Gln Lys Arg Ile Ile Ile Val Phe His Cys
    290                 295                 300

Glu Phe Ser Ser Glu Arg Gly Pro Arg Met Cys Arg Cys Leu Arg Glu
305                 310                 315                 320

Glu Asp Arg Ser Leu Asn Gln Tyr Pro Ala Leu Tyr Tyr Pro Glu Leu
                325                 330                 335

Tyr Ile Leu Lys Gly Gly Tyr Arg Asp Phe Phe Pro Glu Tyr Met Glu
            340                 345                 350

Leu Cys Glu Pro Gln Ser Tyr Cys Pro Met His His Gln Asp His Lys
        355                 360                 365

Thr Glu Leu Leu Arg Cys Arg Ser Gln Ser Lys Val Gln Glu Gly Glu
    370                 375                 380

Arg Gln Leu Arg Glu Gln Ile Ala Leu Leu Val Lys Asp Met Ser Pro
385                 390                 395                 400
```

<210> SEQ ID NO 816
<211> LENGTH: 3318
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 816

```
gtgatgcgta gttccggctg ccggttgaca tgaagaagca gcagcggcta gggcggcggt     60
agctgcaggg gtcggggatt gcagcgggcc tcggggctaa gagcgcgacg cggcctagag    120
cggcagacgg cgcagtgggc cgagaaggag gcgcagcagc cgccctggcc cgtcatggag    180
atggaaaagg agttcgagca gatcgacaag tccgggagct gggcggccat ttaccaggat    240
atccgacatg aagccagtga cttcccatgt agagtggcca agcttcctaa gaacaaaaac    300
cgaaataggt acagagacgt cagtcccttt gaccatagtc ggattaaact acatcaagaa    360
gataatgact atatcaacgc tagtttgata aaaatggaag aagcccaaag gagttacatt    420
cttacccagg gccctttgcc taacacatgc ggtcactttt gggagatggt gtgggagcag    480
aaaagcaggg gtgtcgtcat gctcaacaga gtgatggaga aaggttcgtt aaaatgcgca    540
caatactggc cacaaaaaga agaaaaagag atgatctttg aagacacaaa tttgaaatta    600
acattgatct ctgaagatat caagtcatat tatacagtgc gacagctaga attggaaaac    660
cttacaaccc aagaaactcg agagatctta catttccact ataccacatg gcctgacttt    720
ggagtccctg aatcaccagc ctcattcttg aactttcttt tcaaagtccg agagtcaggg    780
tcactcagcc cggagcacgg gcccgttgtg gtgcactgca gtgcaggcat cggcaggtct    840
ggaaccttct gtctggctga tacctgcctc ttgctgatgg acaagaggaa agacccttct    900
tccgttgata tcaagaaagt gctgttagaa atgaggaagt ttcggatggg gctgatccag    960
acagccgacc agctgcgctt ctcctacctg gctgtgatcg aaggtgccaa attcatcatg   1020
ggggactctt ccgtgcagga tcagtggaag gagctttccc acgaggacct ggagccccca   1080
cccgagcata tccccccacc tccccggcca cccaaacgaa tcctggagcc acacaatggg   1140
aaatgcaggg agttcttccc aaatcaccag tgggtgaagg aagagaccca ggaggataaa   1200
gactgcccca tcaaggaaga aaaaggaagc cccttaaatg ccgcacccta cggcatcgaa   1260
agcatgagtc aagacactga agttagaagt cgggtcgtgg ggggaagtct tcgaggtgcc   1320
```

-continued

```
caggctgcct ccccagccaa aggggagccg tcactgcccg agaaggacga ggaccatgca   1380
ctgagttact ggaagccctt cctggtcaac atgtgcgtgg ctacggtcct cacggccggc   1440
gcttacctct gctacaggtt cctgttcaac agcaacacat agcctgaccc tcctccactc   1500
cacctccacc cactgtccgc ctctgcccgc agagcccacg cccgactagc aggcatgccg   1560
cggtaggtaa gggccgccgg accgcgtaga gagccgggcc ccggacggac gttggttctg   1620
cactaaaacc catcttcccc ggatgtgtgt ctcacccctc atccttttac tttttgcccc   1680
ttccactttg agtaccaaat ccacaagcca ttttttgagg agagtgaaag agagtaccat   1740
gctggcggcg cagagggaag gggcctacac ccgtcttggg gctcgcccca cccagggctc   1800
cctcctggag catcccaggc gggcggcacg ccaacagccc ccccсttgaa tctgcaggga   1860
gcaactctcc actccatatt tatttaaaca attttttccc caaaggcatc catagtgcac   1920
tagcattttc ttgaaccaat aatgtattaa aatttttga tgtcagcctt gcatcaaggg   1980
ctttatcaaa aagtacaata ataaatcctc aggtagtact gggaatggaa ggctttgcca   2040
tgggcctgct gcgtcagacc agtactggga aggaggacgg ttgtaagcag ttgttattta   2100
gtgatattgt gggtaacgtg agaagataga acaatgctat aatatataat gaacacgtgg   2160
gtatttaata agaaacatga tgtgagatta ctttgtcccg cttattctcc tccctgttat   2220
ctgctagatc tagttctcaa tcactgctcc cccgtgtgta ttagaatgca tgtaaggtct   2280
tcttgtgtcc tgatgaaaaa tatgtgcttg aaatgagaaa ctttgatctc tgcttactaa   2340
tgtgccccat gtccaagtcc aacctgcctg tgcatgacct gatcattaca tggctgtggt   2400
tcctaagcct gttgctgaag tcattgtcgc tcagcaatag ggtgcagttt tccaggaata   2460
ggcatttgcc taattcctgg catgacactc tagtgacttc ctggtgaggc ccagcctgtc   2520
ctggtacagc agggtcttgc tgtaactcag acattccaag ggtatgggaa gccatattca   2580
cacctcacgc tctggacatg atttagggaa gcagggacac cccccgcccc ccacctttgg   2640
gatcagcctc cgccattcca agtcaacact cttcttgagc agaccgtgat ttggaagaga   2700
ggcacctgct ggaaaccaca cttcttgaaa cagcctgggt gacggtcctt taggcagcct   2760
gccgccgtct ctgtcccggt tcaccttgcc gagagaggcg cgtctgcccc accctcaaac   2820
cctgtggggc ctgatggtgc tcacgactct tcctgcaaag ggaactgaag acctccacat   2880
taagtggctt tttaacatga aaaacacggc agctgtagct cccgagctac tctcttgcca   2940
gcattttcac attttgcctt tctcgtggta gaagccagta cagagaaatt ctgtggtggg   3000
aacattcgag gtgtcaccct gcagagctat ggtgaggtgt ggataaggct taggtgccag   3060
gctgtaagca ttctgagctg ggcttgttgt ttttaagtcc tgtatatgta tgtagtagtt   3120
tgggtgtgta tatatagtag catttcaaaa tggacgtact ggtttaacct cctatccttg   3180
gagagcagct ggctctccac cttgttacac attatgttag agaggtagcg agctgctctg   3240
ctatatgcct taagccaata tttactcatc aggtcattat tttttacaat ggccatggaa   3300
taaaccattt ttacaaaa                                                  3318
```

<210> SEQ ID NO 817
<211> LENGTH: 435
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 817

-continued

```
Met Glu Met Glu Lys Glu Phe Glu Gln Ile Asp Lys Ser Gly Ser Trp
1               5                   10                  15

Ala Ala Ile Tyr Gln Asp Ile Arg His Glu Ala Ser Asp Phe Pro Cys
            20                  25                  30

Arg Val Ala Lys Leu Pro Lys Asn Lys Asn Arg Asn Arg Tyr Arg Asp
        35                  40                  45

Val Ser Pro Phe Asp His Ser Arg Ile Lys Leu His Gln Glu Asp Asn
    50                  55                  60

Asp Tyr Ile Asn Ala Ser Leu Ile Lys Met Glu Glu Ala Gln Arg Ser
65                  70                  75                  80

Tyr Ile Leu Thr Gln Gly Pro Leu Pro Asn Thr Cys Gly His Phe Trp
                85                  90                  95

Glu Met Val Trp Glu Gln Lys Ser Arg Gly Val Val Met Leu Asn Arg
            100                 105                 110

Val Met Glu Lys Gly Ser Leu Lys Cys Ala Gln Tyr Trp Pro Gln Lys
        115                 120                 125

Glu Glu Lys Glu Met Ile Phe Glu Asp Thr Asn Leu Lys Leu Thr Leu
    130                 135                 140

Ile Ser Glu Asp Ile Lys Ser Tyr Tyr Thr Val Arg Gln Leu Glu Leu
145                 150                 155                 160

Glu Asn Leu Thr Thr Gln Glu Thr Arg Glu Ile Leu His Phe His Tyr
                165                 170                 175

Thr Thr Trp Pro Asp Phe Gly Val Pro Glu Ser Pro Ala Ser Phe Leu
            180                 185                 190

Asn Phe Leu Phe Lys Val Arg Glu Ser Gly Ser Leu Ser Pro Glu His
        195                 200                 205

Gly Pro Val Val Val His Cys Ser Ala Gly Ile Gly Arg Ser Gly Thr
    210                 215                 220

Phe Cys Leu Ala Asp Thr Cys Leu Leu Leu Met Asp Lys Arg Lys Asp
225                 230                 235                 240

Pro Ser Ser Val Asp Ile Lys Lys Val Leu Leu Glu Met Arg Lys Phe
                245                 250                 255

Arg Met Gly Leu Ile Gln Thr Ala Asp Gln Leu Arg Phe Ser Tyr Leu
            260                 265                 270

Ala Val Ile Glu Gly Ala Lys Phe Ile Met Gly Asp Ser Ser Val Gln
        275                 280                 285

Asp Gln Trp Lys Glu Leu Ser His Glu Asp Leu Glu Pro Pro Pro Glu
    290                 295                 300

His Ile Pro Pro Pro Pro Arg Pro Pro Lys Arg Ile Leu Glu Pro His
305                 310                 315                 320

Asn Gly Lys Cys Arg Glu Phe Phe Pro Asn His Gln Trp Val Lys Glu
                325                 330                 335

Glu Thr Gln Glu Asp Lys Asp Cys Pro Ile Lys Glu Glu Lys Gly Ser
            340                 345                 350

Pro Leu Asn Ala Ala Pro Tyr Gly Ile Glu Ser Met Ser Gln Asp Thr
        355                 360                 365

Glu Val Arg Ser Arg Val Val Gly Gly Ser Leu Arg Gly Ala Gln Ala
    370                 375                 380

Ala Ser Pro Ala Lys Gly Glu Pro Ser Leu Pro Glu Lys Asp Glu Asp
385                 390                 395                 400

His Ala Leu Ser Tyr Trp Lys Pro Phe Leu Val Asn Met Cys Val Ala
```

-continued

```
            405                 410                 415

Thr Val Leu Thr Ala Gly Ala Tyr Leu Cys Tyr Arg Phe Leu Phe Asn
        420                 425                 430

Ser Asn Thr
        435


<210> SEQ ID NO 818
<211> LENGTH: 2346
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 818

gaattcggga tccttttgca cattcctagt tagcagtgca tactcatcag actggagatg     60

tttaatgaca tcagggaacc aaacggacaa cccatagtac ccgaagacag ggtgaaccag    120

acaatcgtaa gcttgatggt gttttccctg actgggtagt tgaagcatct catgaatgtc    180

agccaaattc cgtacagttc ggtgcggatc cgaacgaaac acctcctgta ccaggttccc    240

gtgtcgctct caatttcaat cagctcatct atttgtttgg gagtcttgat tttatttacc    300

gtgaagacct tctctggctg gccccgggct ctcatgttgg tgtcatgaat taacttcaga    360

atcatccagg cttcatcatg ttttcccacc tccagcaaga accgagggct ttctggcatg    420

aaggtgagag ccaccacaga ggagacgcat gggagcgcac agacgatgac gaagacgcgc    480

cacgtgtgga actggtaggc tgaacccatg ctgaagctcc acccgtagtg ggaatgatg     540

gcccaggcat ggcggaggct agatgccgcc aatcatccag aacatgcaga agccgctgct    600

ggggagcttg ggctgcggt ggtggcgggt gacgggcttc gggacgcgga gcgacgcggc     660

ctagcgcggc ggacggccgt gggaactcgg gcagccgacc cgtcccgcca tggagatgga    720

gaaggagttc gaggagatcg acaaggctgg gaactgggcg gctatttacc aggacattcg    780

acatgaagcc agcgacttcc catgcaaagt cgcgaagctt cctaagaaca aaaaccggaa    840

caggtaccga gatgtcagcc cttttgacca cagtcggatt aaattgcacc aggaagataa    900

tgactatatc aatgccagct tgataaaaat ggaagaagcc cagaggagct atattctcac    960

ccagggccct ttaccaaaca catgtgggca cttctgggag atggtgtggg agcagaagag   1020

caggggcgtg gtcatgctca accgcatcat ggagaaaggc tcgttaaaat gtgcccagta   1080

ttggccacag caagaagaaa aggagatggt ctttgatgac acaggtttga agttgacact   1140

aatctctgaa gatgtcaagt catattacac agtacgacag ttggagttgg aaaacctgac   1200

taccaaggag actcgagaga tcctgcattt ccactacacc acatggcctg actttggagt   1260

ccccgagtca ccggcttctt tcctcaattt ccttttcaaa gtccgagagt caggctcact   1320

cagcctggag catggcccca ttgtggtcca ctgcagcgcc ggcatcggga ggtcagggac   1380

cttctgtctg gctgacacct gcctcttact gatggacaag aggaaagacc catcttccgt   1440

ggacatcaag aaagtactgc tggagatgcg caggttccgc atggggctca tccagactgc   1500

cgaccagctg cgcttctcct acctggctgt catcgagggc gccaagttca tcatgggcga   1560

ctcgtcagtg caggatcagt ggaaggagct ctcccgggag gatctagacc ttccacccga   1620

gcacgtgccc ccacctcccc ggccacccaa acgcacactg gagcctcaca acgggaagtg   1680

caaggagctc ttctccagcc accagtgggt gagcgaggag acctgtgggg atgaagacag   1740

cctggccaga gaggaaggca gagcccagtc aagtgccatg cacagcgtga gcagcatgag   1800
```

-continued

```
tccagacact gaagttagga gacggatggt gggtggaggt cttcaaagtg ctcaggcgtc   1860

tgtccccacc gaggaagagc tgtcctccac tgaggaggaa cacaaggcac attggccaag   1920

tcactggaag cccttcctgg tcaatgtgtg catggccacg ctcctggcca ccggcgcgta   1980

cttgtgctac cgggtgtgtt ttcactgaca gactgggagg cactgccact gcccagctta   2040

ggatgcggtc tgcggcgtct gacctggtgt agagggaaca acaactcgca agcctgctct   2100

ggaactggaa gggcctgccc caggagggta ttagtgcact gggctttgaa ggagcccctg   2160

gtcccacgaa cagagtctaa tctcagggcc ttaacctgtt caggagaagt agaggaaatg   2220

ccaaatactc ttcttgctct cacctcactc ctcccctttc tctgattcat ttgtttttgg   2280

aaaaaaaaaa aaaaagaatt acaacacatt gttgttttta acatttataa aggcaggccc   2340

gaattc                                                              2346
```

<210> SEQ ID NO 819
<211> LENGTH: 432
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 819

```
Met Glu Met Glu Lys Glu Phe Glu Glu Ile Asp Lys Ala Gly Asn Trp
1               5                   10                  15

Ala Ala Ile Tyr Gln Asp Ile Arg His Glu Ala Ser Asp Phe Pro Cys
            20                  25                  30

Lys Val Ala Lys Leu Pro Lys Asn Lys Asn Arg Asn Arg Tyr Arg Asp
        35                  40                  45

Val Ser Pro Phe Asp His Ser Arg Ile Lys Leu His Gln Glu Asp Asn
    50                  55                  60

Asp Tyr Ile Asn Ala Ser Leu Ile Lys Met Glu Glu Ala Gln Arg Ser
65                  70                  75                  80

Tyr Ile Leu Thr Gln Gly Pro Leu Pro Asn Thr Cys Gly His Phe Trp
                85                  90                  95

Glu Met Val Trp Glu Gln Lys Ser Arg Gly Val Val Met Leu Asn Arg
            100                 105                 110

Ile Met Glu Lys Gly Ser Leu Lys Cys Ala Gln Tyr Trp Pro Gln Gln
        115                 120                 125

Glu Glu Lys Glu Met Val Phe Asp Asp Thr Gly Leu Lys Leu Thr Leu
    130                 135                 140

Ile Ser Glu Asp Val Lys Ser Tyr Tyr Thr Val Arg Gln Leu Glu Leu
145                 150                 155                 160

Glu Asn Leu Thr Thr Lys Glu Thr Arg Glu Ile Leu His Phe His Tyr
                165                 170                 175

Thr Thr Trp Pro Asp Phe Gly Val Pro Glu Ser Pro Ala Ser Phe Leu
            180                 185                 190

Asn Phe Leu Phe Lys Val Arg Glu Ser Gly Ser Leu Ser Leu Glu His
        195                 200                 205

Gly Pro Ile Val Val His Cys Ser Ala Gly Ile Gly Arg Ser Gly Thr
    210                 215                 220

Phe Cys Leu Ala Asp Thr Cys Leu Leu Leu Met Asp Lys Arg Lys Asp
225                 230                 235                 240

Pro Ser Ser Val Asp Ile Lys Lys Val Leu Leu Glu Met Arg Arg Phe
                245                 250                 255
```

-continued

```
Arg Met Gly Leu Ile Gln Thr Ala Asp Gln Leu Arg Phe Ser Tyr Leu
            260                 265                 270

Ala Val Ile Glu Gly Ala Lys Phe Ile Met Gly Asp Ser Ser Val Gln
        275                 280                 285

Asp Gln Trp Lys Glu Leu Ser Arg Glu Asp Leu Asp Leu Pro Pro Glu
    290                 295                 300

His Val Pro Pro Pro Pro Arg Pro Pro Lys Arg Thr Leu Glu Pro His
305                 310                 315                 320

Asn Gly Lys Cys Lys Glu Leu Phe Ser Ser His Gln Trp Val Ser Glu
                325                 330                 335

Glu Thr Cys Gly Asp Glu Asp Ser Leu Ala Arg Glu Glu Gly Arg Ala
            340                 345                 350

Gln Ser Ser Ala Met His Ser Val Ser Ser Met Ser Pro Asp Thr Glu
        355                 360                 365

Val Arg Arg Arg Met Val Gly Gly Gly Leu Gln Ser Ala Gln Ala Ser
    370                 375                 380

Val Pro Thr Glu Glu Glu Leu Ser Ser Thr Glu Glu Glu His Lys Ala
385                 390                 395                 400

His Trp Pro Ser His Trp Lys Pro Phe Leu Val Asn Val Cys Met Ala
                405                 410                 415

Thr Leu Leu Ala Thr Gly Ala Tyr Leu Cys Tyr Arg Val Cys Phe His
            420                 425                 430
```

<210> SEQ ID NO 820
<211> LENGTH: 4127
<212> TYPE: DNA
<213> ORGANISM: Rattus novegicus

<400> SEQUENCE: 820

```
agccgctgct ggggaggttg gggctgaggt ggtggcgggc gacgggcctc gagacgcgga     60

gcgacgcggc ctagcgcggc ggacggccga gggaactcgg gcagtcgtcc cgtcccgcca    120

tggaaatgga gaaggaattc gagcagatcg ataaggctgg gaactgggcg gctatttacc    180

aggatattcg acatgaagcc agtgacttcc catgcagaat agcgaaactt cctaagaaca    240

aaaaccggaa caggtaccga gatgtcagcc cttttgacca cagtcggatt aaattgcatc    300

aggaagataa tgactatatc aatgccagct tgataaaaat ggaggaagcc cagaggagct    360

atatcctcac ccagggccct ttaccaaaca cgtgcgggca cttctgggag atggtgtggg    420

agcagaagag caggggcgtg gtcatgctca accgcatcat ggagaaaggc tcgttaaaat    480

gtgcccagta ttggccacag aaagaagaaa aagagatggt cttcgatgac accaatttga    540

agctgacact gatctctgaa gatgtcaagt catattacac agtacggcag ttggagttgg    600

agaacctggc tacccaggag gctcgagaga tcctgcattt ccactacacc acctggcctg    660

actttggagt ccctgagtca cctgcctctt tcctcaattt cctattcaaa gtccgagagt    720

caggctcact cagcccagag cacggcccca ttgtggtcca ctgcagtgct ggcattggca    780

ggtcagggac cttctgcctg gctgacacct gcctcttact gatggacaag aggaaagacc    840

cgtcctctgt ggacatcaag aaagtgctgt tggagatgcg caggttccgc atggggctca    900

tccagacggc cgaccaactg cgcttctcct acctggctgt gatcgagggt gcaaagttca    960

tcatgggcga ctcgtcagtg caggatcagt ggaaggagct ttcccatgaa gacctggagc   1020

ctccccctga gcacgtgccc ccacctcccc ggccacccaa acgcacattg gagcctcaca   1080
```

-continued

```
atggcaagtg caaggagctc ttctccaacc accagtgggt gagcgaggag agctgtgagg   1140
atgaggacat cctggccaga gaggaaagca gagccccctc aattgctgtg cacagcatga   1200
gcagtatgag tcaagacact gaagttagga aacggatggt gggtggaggt cttcaaagtg   1260
ctcaggcatc tgtccccact gaggaagagc tgtccccaac cgaggaggaa caaaaggcac   1320
acaggccagt tcactggaag cccttcctgg tcaacgtgtg catggccacg gccctggcga   1380
ctggcgcgta cctctgttac cgggtatgtt ttcactgaca gactgctgtg aggcatgagc   1440
gtggtgggcg ctgccactgc ccaggttagg atttggtctg cggcgtctaa cctggtgtag   1500
aagaaacaac agcttacaag cctgtggtgg aactggaagg gccagcccca ggaggggcat   1560
ctgtgcactg ggctttgaag gagcccctgg tcccaagaac agagtctaat ctcagggcct   1620
taacctgttc aggagaagta gaggaaatgc caaatactct tcttgctctc acctcactcc   1680
tcccctttct ctggttcgtt tgtttttgga aaaaaaaaaa aaagaattac aacacattgt   1740
tgtttttaac atttataaag gcaggttttt gttattttta gagaaaacaa aagatgctag   1800
gcactggtga gattctcttg tgccctttgg catgtgatca gattcacgat ttacgtttat   1860
ttccgggga gggtcccacc tgtcaggact gtaaagttcc tgctggcttg gtcagccccc   1920
ccaccccccc accccgagct tgcaggtgcc ctgctgtgag gagagcagca gcagaggctg   1980
cccctggaca gaagcccagc tctgcttccc tcaggtgtcc ctgcgtttcc atcctccttc   2040
tttgtgaccg ccatcttgca gatgacccag tcctcagcac cccacccctg cagatgggtt   2100
tctccgaggg cctgcctcag ggtcatcaga ggttggctgc cagcttagag ctggggcttc   2160
catttgattg gaaagtcatt actattctat gtagaagcca ctccactgag gtgtaaagca   2220
agactcataa aggaggagcc ttggtgtcat ggaagtcact ccgcgcgcag gacctgtaac   2280
aacctctgaa acactcagtc ctgctgcagt gacgtccttg aaggcatcag acagatgatt   2340
tgcagactgc caagacttgt cctgagccgt gatttttaga gtctggactc atgaaacacc   2400
gccgagcgct tactgtgcag cctctgatgc tggttggctg aggctgcggg gaggtggaca   2460
ctgtgggtgc atccagtgca gttgcttttg tgcagttggg tccagcagca cagcccgcac   2520
tccagcctca gctgcaggcc acagtggcca tggaggccgc cagagcgagc tggggtggat   2580
gcttgttcac ttggagcagc cttcccagga cgtgcagctc ccttcctgct ttgtccttct   2640
gcttccttcc ctggagtagc aagcccacga gcaatcgtga ggggtgtgag ggagctgcag   2700
aggcatcaga gtggcctgca gcggcgtgag gcccccttccc ctccgacacc cccctccaga   2760
ggagccgctc cactgttatt tattcacttt gcccacagac accccctgagt gagcacaccc   2820
tgaaactgac cgtgtaaggt gtcagcctgc acccaggacc gtcaggtgca gcaccgggtc   2880
agtcctaggg ttgaggtagg actgacacag ccactgtgtg gctggtgctg gggcaggggc   2940
aggagctgag ggtcttagaa gcaatcttca ggaacagaca acagtggtga catgtaaagt   3000
ccctgtggct actgatgaca tgtgtaggat gaaggctggc ctttctccca tgactttcta   3060
gatcccgttc cccgtctgct ttccctgtga gttagaaaac acacaggctc ctgtcctggt   3120
ggtgccgtgt gcttgacatg ggaaacttag atgcctgctc actggcgggc acctcggcat   3180
cgccaccact cagagtgaga gcagtgctgt ccagtgccga ggccgcctga ctcccggcag   3240
gactcttcag gctctggcct gccccagcac accccgctgg atctcagaca ttccacaccc   3300
acacctcatt ccctggacac ttgggcaagc aggcccgccc ttccacctct ggggtcagcc   3360
```

-continued

```
cctccattcc gagttcacac tgctctggag caggccagga ccggaagcaa ggcagctggt   3420
gaggagcacc ctcctgggaa cagtgtaggt gacagtcctg agagtcagct tgctagcgct   3480
gctggcacca gtcaccttgc tcagaagtgt gtggctcttg aggctgaaga gactgatgat   3540
ggtgctcatg actcttctgt gaggggaact tgaccttcac attgggtggc tttttttaaa   3600
ataagcgaag gcagctggaa ctccagtctg cctcttgcca gcacttcaca ttttgccttt   3660
cacccagaga agccagcaca gagccactgg ggaaggcgat ggccttgcct gcacaggctg   3720
aggagatggc tcagccggcg tccaggctgt gtctggagca gggggtgcac agcagcctca   3780
caggtggggg cctcagagca ggcgctgccc tgtcccctgc cccgctggag gcagcaaagc   3840
tgctgcatgc cttaagtcaa tacttactca gcagggcgct ctcgttctct ctctctctct   3900
ctctctctct ctctctctct ctctctctct ctctaaatgg ccatagaata aaccatttta   3960
caaaaataaa agccaacaac aaagtgctct ggaatagcac ctttgcagga gcgggggggtg   4020
tctcagggtc ttctgtgacc tcaccgaact gtccgactgc accgtttcca acttgtgtct   4080
cactaatggg tctgcattag ttgcaacaat aaatgttttt aaagaac                 4127
```

<210> SEQ ID NO 821
<211> LENGTH: 432
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 821

```
Met Glu Met Glu Lys Glu Phe Glu Gln Ile Asp Lys Ala Gly Asn Trp
1               5                   10                  15

Ala Ala Ile Tyr Gln Asp Ile Arg His Glu Ala Ser Asp Phe Pro Cys
            20                  25                  30

Arg Ile Ala Lys Leu Pro Lys Asn Lys Asn Arg Asn Arg Tyr Arg Asp
        35                  40                  45

Val Ser Pro Phe Asp His Ser Arg Ile Lys Leu His Gln Glu Asp Asn
    50                  55                  60

Asp Tyr Ile Asn Ala Ser Leu Ile Lys Met Glu Glu Ala Gln Arg Ser
65                  70                  75                  80

Tyr Ile Leu Thr Gln Gly Pro Leu Pro Asn Thr Cys Gly His Phe Trp
                85                  90                  95

Glu Met Val Trp Glu Gln Lys Ser Arg Gly Val Val Met Leu Asn Arg
            100                 105                 110

Ile Met Glu Lys Gly Ser Leu Lys Cys Ala Gln Tyr Trp Pro Gln Lys
        115                 120                 125

Glu Glu Lys Glu Met Val Phe Asp Asp Thr Asn Leu Lys Leu Thr Leu
    130                 135                 140

Ile Ser Glu Asp Val Lys Ser Tyr Tyr Thr Val Arg Gln Leu Glu Leu
145                 150                 155                 160

Glu Asn Leu Ala Thr Gln Glu Ala Arg Glu Ile Leu His Phe His Tyr
                165                 170                 175

Thr Thr Trp Pro Asp Phe Gly Val Pro Glu Ser Pro Ala Ser Phe Leu
            180                 185                 190

Asn Phe Leu Phe Lys Val Arg Glu Ser Gly Ser Leu Ser Pro Glu His
        195                 200                 205

Gly Pro Ile Val Val His Cys Ser Ala Gly Ile Gly Arg Ser Gly Thr
    210                 215                 220
```

-continued

```
Phe Cys Leu Ala Asp Thr Cys Leu Leu Leu Met Asp Lys Arg Lys Asp
225                 230                 235                 240

Pro Ser Ser Val Asp Ile Lys Lys Val Leu Leu Glu Met Arg Arg Phe
                245                 250                 255

Arg Met Gly Leu Ile Gln Thr Ala Asp Gln Leu Arg Phe Ser Tyr Leu
            260                 265                 270

Ala Val Ile Glu Gly Ala Lys Phe Ile Met Gly Asp Ser Ser Val Gln
        275                 280                 285

Asp Gln Trp Lys Glu Leu Ser His Glu Asp Leu Glu Pro Pro Pro Glu
    290                 295                 300

His Val Pro Pro Pro Pro Arg Pro Pro Lys Arg Thr Leu Glu Pro His
305                 310                 315                 320

Asn Gly Lys Cys Lys Glu Leu Phe Ser Asn His Gln Trp Val Ser Glu
                325                 330                 335

Glu Ser Cys Glu Asp Glu Asp Ile Leu Ala Arg Glu Glu Ser Arg Ala
            340                 345                 350

Pro Ser Ile Ala Val His Ser Met Ser Ser Met Ser Gln Asp Thr Glu
        355                 360                 365

Val Arg Lys Arg Met Val Gly Gly Gly Leu Gln Ser Ala Gln Ala Ser
    370                 375                 380

Val Pro Thr Glu Glu Glu Leu Ser Pro Thr Glu Glu Glu Gln Lys Ala
385                 390                 395                 400

His Arg Pro Val His Trp Lys Pro Phe Leu Val Asn Val Cys Met Ala
                405                 410                 415

Thr Ala Leu Ala Thr Gly Ala Tyr Leu Cys Tyr Arg Val Cys Phe His
            420                 425                 430
```

<210> SEQ ID NO 822
<211> LENGTH: 2287
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 822

```
gggggggcctg agcctctccg ccggcgcagg ctctgctcgc gccagctcgc tcccgcagcc      60
atgcccacca ccatcgagcg ggagttcgaa gagttggata ctcagcgtcg ctggcagccg     120
ctgtacttgg aaattcgaaa tgagtcccat gactatcctc atagagtggc caagtttcca     180
gaaaacagaa atcgaaacag atacagagat gtaagcccat atgatcacag tcgtgttaaa     240
ctgcaaaatg ctgagaatga ttatattaat gccagtttag ttgacataga agaggcacaa     300
aggagttaca tcttaacaca gggtccactt cctaacacat gctgccattt ctggcttatg     360
gtttggcagc agaagaccaa agcagttgtc atgctgaacc gcattgtgga gaaagaatcg     420
gttaaatgtg cacagtactg gccaacagat gaccaagaga tgctgtttaa agaaacagga     480
ttcagtgtga agctcttgtc agaagatgtg aagtcgtatt atacagtaca tctactacaa     540
ttagaaaata tcaatagtgg tgaaaccaga acaatatctc actttcatta tactacctgg     600
ccagattttg gagtccctga atcaccagct tcatttctca atttcttgtt taaagtgaga     660
gaatctggct ccttgaaccc tgaccatggg cctgcggtga tccactgtag tgcaggcatt     720
gggcgctctg gcaccttctc tctggtagac acttgtcttg ttttgatgga aaaaggagat     780
gatattaaca taaaacaagt gttactgaac atgagaaaat accgaatggg tcttattcag     840
```

-continued

```
accccagatc aactgagatt ctcatacatg gctataatag aaggagcaaa atgtataaag     900

ggagattcta gtatacagaa acgatggaaa gaactttcta aggaagactt atctcctgcc     960

tttgatcatt caccaaacaa aataatgact gaaaaataca atgggaacag aataggtcta    1020

gaagaagaaa aactgacagg tgaccgatgt acaggacttt cctctaaaat gcaagataca    1080

atggaggaga acagtgagag tgctctacgg aaacgtattc gagaggacag aaaggccacc    1140

acagctcaga aggtgcagca gatgaaacag aggctaaatg agaatgaacg aaaaagaaaa    1200

aggtggttat attggcaacc tattctcact aagatggggt ttatgtcagt cattttggtt    1260

ggcgcttttg ttggctggag actgtttttt cagcaaaatg ccctataaac aattaatttt    1320

gcccagcaag cttctgcact agtaactgac agtgctacat taatcatagg ggtttgtctg    1380

cagcaaacgc ctcatatccc aaaaacggtg cagtagaata gacatcaacc agataagtga    1440

tatttacagt cacaagccca acatctcagg actcttgact gcaggttcct ctgaacccca    1500

aactgtaaat ggctgtctaa aataaagaca ttcatgtttg ttaaaaactg gtaaattttg    1560

caactgtatt catacatgtc aaacacagta tttcacctga ccaacattga gatatccttt    1620

atcacaggat ttgtttttgg aggctatctg gattttaacc tgcacttgat ataagcaata    1680

aatattgtgg ttttatctac gttattggaa agaaaatgac atttaaataa tgtgtgtaat    1740

gtataatgta ctattgacat gggcatcaac acttttattc ttaagcattt cagggtaaat    1800

atattttata agtatctatt taatcttttg tagttaactg tactttttaa gagctcaatt    1860

tgaaaaatct gttactaaaa aaaaaaattg tatgtcgatt gaattgtact ggatacattt    1920

tccatttttc taaaaagaag tttgatatga gcagttagaa gttggaataa gcaatttcta    1980

ctatatattg catttctttt atgttttaca gttttcccca ttttaaaaag aaaagcaaac    2040

aaagaaacaa aagtttttcc taaaaatatc tttgaaggaa aattctcctt actgggatag    2100

tcaggtaaac agttggtcaa gactttgtaa agaaattggt ttctgtaaat cccattattg    2160

atatgtttat ttttcatgaa aatttcaatg tagttggggt agattatgat ttaggaagca    2220

aaagtaagaa gcagcatttt atgattcata atttcagttt actagactga agttttgaag    2280

taaaccc                                                              2287
```

<210> SEQ ID NO 823
<211> LENGTH: 415
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 823

```
Met Pro Thr Thr Ile Glu Arg Glu Phe Glu Glu Leu Asp Thr Gln Arg
1               5                   10                  15

Arg Trp Gln Pro Leu Tyr Leu Glu Ile Arg Asn Glu Ser His Asp Tyr
            20                  25                  30

Pro His Arg Val Ala Lys Phe Pro Glu Asn Arg Asn Arg Asn Arg Tyr
        35                  40                  45

Arg Asp Val Ser Pro Tyr Asp His Ser Arg Val Lys Leu Gln Asn Ala
    50                  55                  60

Glu Asn Asp Tyr Ile Asn Ala Ser Leu Val Asp Ile Glu Glu Ala Gln
65                  70                  75                  80

Arg Ser Tyr Ile Leu Thr Gln Gly Pro Leu Pro Asn Thr Cys Cys His
                85                  90                  95
```

-continued

```
Phe Trp Leu Met Val Trp Gln Gln Lys Thr Lys Ala Val Val Met Leu
            100                 105                 110

Asn Arg Ile Val Glu Lys Glu Ser Val Lys Cys Ala Gln Tyr Trp Pro
        115                 120                 125

Thr Asp Asp Gln Glu Met Leu Phe Lys Glu Thr Gly Phe Ser Val Lys
    130                 135                 140

Leu Leu Ser Glu Asp Val Lys Ser Tyr Tyr Thr Val His Leu Leu Gln
145                 150                 155                 160

Leu Glu Asn Ile Asn Ser Gly Glu Thr Arg Thr Ile Ser His Phe His
                165                 170                 175

Tyr Thr Thr Trp Pro Asp Phe Gly Val Pro Glu Ser Pro Ala Ser Phe
            180                 185                 190

Leu Asn Phe Leu Phe Lys Val Arg Glu Ser Gly Ser Leu Asn Pro Asp
        195                 200                 205

His Gly Pro Ala Val Ile His Cys Ser Ala Gly Ile Gly Arg Ser Gly
    210                 215                 220

Thr Phe Ser Leu Val Asp Thr Cys Leu Val Leu Met Glu Lys Gly Asp
225                 230                 235                 240

Asp Ile Asn Ile Lys Gln Val Leu Leu Asn Met Arg Lys Tyr Arg Met
                245                 250                 255

Gly Leu Ile Gln Thr Pro Asp Gln Leu Arg Phe Ser Tyr Met Ala Ile
            260                 265                 270

Ile Glu Gly Ala Lys Cys Ile Lys Gly Asp Ser Ser Ile Gln Lys Arg
        275                 280                 285

Trp Lys Glu Leu Ser Lys Glu Asp Leu Ser Pro Ala Phe Asp His Ser
    290                 295                 300

Pro Asn Lys Ile Met Thr Glu Lys Tyr Asn Gly Asn Arg Ile Gly Leu
305                 310                 315                 320

Glu Glu Glu Lys Leu Thr Gly Asp Arg Cys Thr Gly Leu Ser Ser Lys
                325                 330                 335

Met Gln Asp Thr Met Glu Glu Asn Ser Glu Ser Ala Leu Arg Lys Arg
            340                 345                 350

Ile Arg Glu Asp Arg Lys Ala Thr Thr Ala Gln Lys Val Gln Gln Met
        355                 360                 365

Lys Gln Arg Leu Asn Glu Asn Glu Arg Lys Arg Lys Arg Trp Leu Tyr
    370                 375                 380

Trp Gln Pro Ile Leu Thr Lys Met Gly Phe Met Ser Val Ile Leu Val
385                 390                 395                 400

Gly Ala Phe Val Gly Trp Arg Leu Phe Phe Gln Gln Asn Ala Leu
                405                 410                 415


<210> SEQ ID NO 824
<211> LENGTH: 2477
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 824

gctcgggcgc cgagtctgcg cgctgacgtc cgacgctcca ggtactttcc ccacggccga      60

cagggcttgg cgtgggggcg gggcgcggcg cgcagcgcgc atgcgccgca gcgccagcgc     120

tctccccgga tcgtgcgggg cctgagcctc tccgccggcg caggctctgc tcgcgccagc     180

tcgctcccgc agccatgccc accaccatcg agcgggagtt cgaagagttg gatactcagc     240
```

-continued

```
gtcgctggca gccgctgtac ttggaaattc gaaatgagtc ccatgactat cctcatagag    300
tggccaagtt tccagaaaac agaaatcgaa acagatacag agatgtaagc ccatatgatc    360
acagtcgtgt taaactgcaa aatgctgaga atgattatat taatgccagt ttagttgaca    420
tagaagaggc acaaaggagt tacatcttaa cacagggtcc acttcctaac acatgctgcc    480
atttctggct tatggtttgg cagcagaaga ccaaagcagt tgtcatgctg aaccgcattg    540
tggagaaaga atcggttaaa tgtgcacagt actggccaac agatgaccaa gagatgctgt    600
ttaaagaaac aggattcagt gtgaagctct tgtcagaaga tgtgaagtcg tattatacag    660
tacatctact acaattagaa aatatcaata gtggtgaaac cagaacaata tctcactttc    720
attatactac ctggccagat tttggagtcc ctgaatcacc agcttcattt ctcaatttct    780
tgtttaaagt gagagaatct ggctccttga accctgacca tgggcctgcg gtgatccact    840
gtagtgcagg cattgggcgc tctggcacct tctctctggt agacacttgt cttgttttga    900
tggaaaaagg agatgatatt aacataaaac aagtgttact gaacatgaga aaataccgaa    960
tgggtcttat tcagacccca gatcaactga gattctcata catggctata atagaaggag   1020
caaaatgtat aaagggagat tctagtatac agaaacgatg gaaagaactt tctaaggaag   1080
acttatctcc tgcctttgat cattcaccaa acaaaataat gactgaaaaa tacaatggga   1140
acagaatagg tctagaagaa gaaaaactga caggtgaccg atgtacagga ctttcctcta   1200
aaatgcaaga tacaatggag gagaacagtg agagtgctct acggaaacgt attcgagagg   1260
acagaaaggc caccacagct cagaaggtgc agcagatgaa acagaggcta aatgagaatg   1320
aacgaaaaag aaaaaggtgg ttatattggc aacctattct cactaagatg ggggtttatgt  1380
cagtcatttt ggttggcgct tttgttggct ggagactgtt ttttcagcaa aatgccctat   1440
aaacaattaa ttttgcccag caagcttctg cactagtaac tgacagtgct acattaatca   1500
taggggtttg tctgcagcaa acgcctcata tcccaaaaac ggtgcagtag aatagacatc   1560
aaccagataa gtgatattta cagtcacaag cccaacatct caggactctt gactgcaggt   1620
tcctctgaac cccaaactgt aaatggctgt ctaaaataaa gacattcatg tttgttaaaa   1680
actggtaaat tttgcaactg tattcataca tgtcaaacac agtatttcac ctgaccaaca   1740
ttgagatatc ctttatcaca ggatttgttt ttggaggcta tctggatttt aacctgcact   1800
tgatataagc aataaatatt gtggttttat ctacgttatt ggaaagaaaa tgacatttaa   1860
ataatgtgtg taatgtataa tgtactattg acatgggcat caacactttt attcttaagc   1920
atttcagggt aaatatattt tataagtatc tatttaatct tttgtagtta actgtacttt   1980
ttaagagctc aatttgaaaa atctgttact aaaaaaaaaa attgtatgtc gattgaattg   2040
tactggatac attttccatt tttctaaaaa gaagtttgat atgagcagtt agaagttgga   2100
ataagcaatt tctactatat attgcatttc ttttatgttt tacagttttc cccattttaa   2160
aaagaaaagc aaacaaagaa acaaaagttt ttcctaaaaa tatctttgaa ggaaaattct   2220
ccttactggg atagtcaggt aaacagttgg tcaagacttt gtaaagaaat tggtttctgt   2280
aaatcccatt attgatatgt ttatttttca tgaaaatttc aatgtagttg gggtagatta   2340
tgatttagga agcaaaagta agaagcagca ttttatgatt cataatttca gtttactaga   2400
ctgaagtttt gaagtaaaca cttttcagtt tctttctact tcaataaata gtatgattat   2460
atgcaaacct taaaaaa                                                  2477
```

-continued

<210> SEQ ID NO 825
<211> LENGTH: 415
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 825

```
Met Pro Thr Thr Ile Glu Arg Glu Phe Glu Glu Leu Asp Thr Gln Arg
1               5                   10                  15

Arg Trp Gln Pro Leu Tyr Leu Glu Ile Arg Asn Glu Ser His Asp Tyr
            20                  25                  30

Pro His Arg Val Ala Lys Phe Pro Glu Asn Arg Asn Arg Asn Arg Tyr
        35                  40                  45

Arg Asp Val Ser Pro Tyr Asp His Ser Arg Val Lys Leu Gln Asn Ala
    50                  55                  60

Glu Asn Asp Tyr Ile Asn Ala Ser Leu Val Asp Ile Glu Glu Ala Gln
65                  70                  75                  80

Arg Ser Tyr Ile Leu Thr Gln Gly Pro Leu Pro Asn Thr Cys Cys His
                85                  90                  95

Phe Trp Leu Met Val Trp Gln Gln Lys Thr Lys Ala Val Val Met Leu
            100                 105                 110

Asn Arg Ile Val Glu Lys Glu Ser Val Lys Cys Ala Gln Tyr Trp Pro
        115                 120                 125

Thr Asp Asp Gln Glu Met Leu Phe Lys Glu Thr Gly Phe Ser Val Lys
    130                 135                 140

Leu Leu Ser Glu Asp Val Lys Ser Tyr Tyr Thr Val His Leu Leu Gln
145                 150                 155                 160

Leu Glu Asn Ile Asn Ser Gly Glu Thr Arg Thr Ile Ser His Phe His
                165                 170                 175

Tyr Thr Thr Trp Pro Asp Phe Gly Val Pro Glu Ser Pro Ala Ser Phe
            180                 185                 190

Leu Asn Phe Leu Phe Lys Val Arg Glu Ser Gly Ser Leu Asn Pro Asp
        195                 200                 205

His Gly Pro Ala Val Ile His Cys Ser Ala Gly Ile Gly Arg Ser Gly
    210                 215                 220

Thr Phe Ser Leu Val Asp Thr Cys Leu Val Leu Met Glu Lys Gly Asp
225                 230                 235                 240

Asp Ile Asn Ile Lys Gln Val Leu Leu Asn Met Arg Lys Tyr Arg Met
                245                 250                 255

Gly Leu Ile Gln Thr Pro Asp Gln Leu Arg Phe Ser Tyr Met Ala Ile
            260                 265                 270

Ile Glu Gly Ala Lys Cys Ile Lys Gly Asp Ser Ser Ile Gln Lys Arg
        275                 280                 285

Trp Lys Glu Leu Ser Lys Glu Asp Leu Ser Pro Ala Phe Asp His Ser
    290                 295                 300

Pro Asn Lys Ile Met Thr Glu Lys Tyr Asn Gly Asn Arg Ile Gly Leu
305                 310                 315                 320

Glu Glu Glu Lys Leu Thr Gly Asp Arg Cys Thr Gly Leu Ser Ser Lys
                325                 330                 335

Met Gln Asp Thr Met Glu Glu Asn Ser Glu Ser Ala Leu Arg Lys Arg
            340                 345                 350

Ile Arg Glu Asp Arg Lys Ala Thr Thr Ala Gln Lys Val Gln Gln Met
        355                 360                 365
```

-continued

```
Lys Gln Arg Leu Asn Glu Asn Glu Arg Lys Arg Lys Arg Trp Leu Tyr
    370                 375                 380

Trp Gln Pro Ile Leu Thr Lys Met Gly Phe Met Ser Val Ile Leu Val
385                 390                 395                 400

Gly Ala Phe Val Gly Trp Arg Leu Phe Phe Gln Gln Asn Ala Leu
                405                 410                 415


&lt;210&gt; SEQ ID NO 826
&lt;211&gt; LENGTH: 1714
&lt;212&gt; TYPE: DNA
&lt;213&gt; ORGANISM: Homo sapiens

&lt;400&gt; SEQUENCE: 826

gctcgggcgc cgagtctgcg cgctgacgtc cgacgctcca ggtactttcc ccacggccga     60

cagggcttgg cgtgggggcg gggcgcggcg cgcagcgcgc atgcgccgca gcgccagcgc    120

tctccccgga tcgtgcgggg cctgagcctc tccgccggcg caggctctgc tcgcgccagc    180

tcgctcccgc agccatgccc accaccatcg agcgggagtt cgaagagttg gatactcagc    240

gtcgctggca gccgctgtac ttggaaattc gaaatgagtc ccatgactat cctcatagag    300

tggccaagtt tccagaaaac agaaatcgaa acagatacag agatgtaagc ccatatgatc    360

acagtcgtgt taaactgcaa aatgctgaga atgattatat taatgccagt ttagttgaca    420

tagaagaggc acaaaggagt tacatcttaa cacagggtcc acttcctaac acatgctgcc    480

atttctggct tatggtttgg cagcagaaga ccaaagcagt tgtcatgctg aaccgcattg    540

tggagaaaga atcggttaaa tgtgcacagt actggccaac agatgaccaa gagatgctgt    600

ttaaagaaac aggattcagt gtgaagctct tgtcagaaga tgtgaagtcg tattatacag    660

tacatctact acaattagaa aatatcaata gtggtgaaac cagaacaata tctcactttc    720

attatactac ctggccagat tttggagtcc ctgaatcacc agcttcattt ctcaatttct    780

tgtttaaagt gagagaatct ggctccttga accctgacca tgggcctgcg gtgatccact    840

gtagtgcagg cattgggcgc tctggcacct tctctctggt agacacttgt cttgttttga    900

tggaaaaagg agatgatatt aacataaaac aagtgttact gaacatgaga aaataccgaa    960

tgggtcttat tcagacccca gatcaactga gattctcata catggctata atagaaggag   1020

caaaatgtat aaagggagat tctagtatac agaaacgatg gaaagaactt tctaaggaag   1080

acttatctcc tgcctttgat cattcaccaa acaaaataat gactgaaaaa tacaatggga   1140

acagaatagg tctagaagaa gaaaaactga caggtgaccg atgtacagga ctttcctcta   1200

aaatgcaaga tacaatggag gagaacagtg agagtgctct acggaaacgt attcgagagg   1260

acagaaaggc caccacagct cagaaggtgc agcagatgaa acagaggcta aatgagaatg   1320

aacgaaaaag aaaaaggcca agattgacag acacctaata ttcatgactt gagaatattc   1380

tgcagctata aattttgaac cattgatgtg caaagcaaga cctgaagccc actccggaaa   1440

ctaaagtgag gctcgctaac cctctagatt gcctcacagt tgtttgttta caaagtaaac   1500

tttacatcca ggggatgaag agcacccacc agcagaagac tttgcagaac ctttaattgg   1560

atgtgttaag tgtttttaat gagtgtatga aatgtagaaa gatgtacaag aaataaatta   1620

ggagagatta ctttgtattg tactgccatt cctactgtat ttttatactt tttggcagca   1680

ttaaatattt ttgttaaata aaaaaaaaaa aaaa                               1714
```

-continued

```
<210> SEQ ID NO 827
<211> LENGTH: 387
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 827

Met Pro Thr Thr Ile Glu Arg Glu Phe Glu Glu Leu Asp Thr Gln Arg
1               5                   10                  15

Arg Trp Gln Pro Leu Tyr Leu Glu Ile Arg Asn Glu Ser His Asp Tyr
            20                  25                  30

Pro His Arg Val Ala Lys Phe Pro Glu Asn Arg Asn Arg Asn Arg Tyr
        35                  40                  45

Arg Asp Val Ser Pro Tyr Asp His Ser Arg Val Lys Leu Gln Asn Ala
    50                  55                  60

Glu Asn Asp Tyr Ile Asn Ala Ser Leu Val Asp Ile Glu Glu Ala Gln
65                  70                  75                  80

Arg Ser Tyr Ile Leu Thr Gln Gly Pro Leu Pro Asn Thr Cys Cys His
                85                  90                  95

Phe Trp Leu Met Val Trp Gln Gln Lys Thr Lys Ala Val Val Met Leu
            100                 105                 110

Asn Arg Ile Val Glu Lys Glu Ser Val Lys Cys Ala Gln Tyr Trp Pro
        115                 120                 125

Thr Asp Asp Gln Glu Met Leu Phe Lys Glu Thr Gly Phe Ser Val Lys
    130                 135                 140

Leu Leu Ser Glu Asp Val Lys Ser Tyr Tyr Thr Val His Leu Leu Gln
145                 150                 155                 160

Leu Glu Asn Ile Asn Ser Gly Glu Thr Arg Thr Ile Ser His Phe His
                165                 170                 175

Tyr Thr Thr Trp Pro Asp Phe Gly Val Pro Glu Ser Pro Ala Ser Phe
            180                 185                 190

Leu Asn Phe Leu Phe Lys Val Arg Glu Ser Gly Ser Leu Asn Pro Asp
        195                 200                 205

His Gly Pro Ala Val Ile His Cys Ser Ala Gly Ile Gly Arg Ser Gly
    210                 215                 220

Thr Phe Ser Leu Val Asp Thr Cys Leu Val Leu Met Glu Lys Gly Asp
225                 230                 235                 240

Asp Ile Asn Ile Lys Gln Val Leu Leu Asn Met Arg Lys Tyr Arg Met
                245                 250                 255

Gly Leu Ile Gln Thr Pro Asp Gln Leu Arg Phe Ser Tyr Met Ala Ile
            260                 265                 270

Ile Glu Gly Ala Lys Cys Ile Lys Gly Asp Ser Ser Ile Gln Lys Arg
        275                 280                 285

Trp Lys Glu Leu Ser Lys Glu Asp Leu Ser Pro Ala Phe Asp His Ser
    290                 295                 300

Pro Asn Lys Ile Met Thr Glu Lys Tyr Asn Gly Asn Arg Ile Gly Leu
305                 310                 315                 320

Glu Glu Glu Lys Leu Thr Gly Asp Arg Cys Thr Gly Leu Ser Ser Lys
                325                 330                 335

Met Gln Asp Thr Met Glu Glu Asn Ser Glu Ser Ala Leu Arg Lys Arg
            340                 345                 350

Ile Arg Glu Asp Arg Lys Ala Thr Thr Ala Gln Lys Val Gln Gln Met
        355                 360                 365
```

-continued

Lys Gln Arg Leu Asn Glu Asn Glu Arg Lys Arg Lys Arg Pro Arg Leu
    370   375   380

Thr Asp Thr
385

<210> SEQ ID NO 828
<211> LENGTH: 1555
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 828

tctccccgga tagagcgggg cccgagcctg tccgctgtgg tagttccgct cggctgcccc 60

gccgccatgt cggcaaccat cgagcgggag ttcgaggaac tggatgctca gtgtcgctgg 120

cagccgttat acttggaaat tcgaaatgaa tcccatgact atcctcatag agtggccaag 180

tttccagaaa acagaaaccg aaacagatac agagatgtaa gcccatatga tcacagtcgt 240

gttaaactgc aaagtactga aaatgattat attaatgcca gcttagttga catagaagag 300

gcacaaagaa gttacatctt aacacagggc ccacttccga acacatgctg ccatttctgg 360

ctcatggtgt ggcagcaaaa gaccaaagca gttgtcatgc taaaccgaac tgtagaaaaa 420

gaatcggtta aatgtgcaca gtactggcca acggatgaca gagaaatggt gtttaaggaa 480

acgggattca gtgtgaagct cttatctgaa gatgtaaaat catattatac agtacatcta 540

ctacagttag aaaatatcaa tactggtgaa acgagaacca tatctcactt ccattatacc 600

acctggccag attttggggt tccagagtca ccagcttcat ttctaaactt cttgtttaaa 660

gttagagaat ctggttgttt gacccctgac catggacctg cagtgatcca ttgcagtgcg 720

ggcatcgggc gctctggcac cttctctctt gtagatacct gtcttgttct gatggaaaaa 780

ggagaggatg ttaatgtgaa acaattatta ctgaatatga gaaagtatcg aatgggactt 840

attcagacac cggaccaact cagattctcc tacatggcca taatagaagg agcaaagtac 900

acaaaaggag attcaaatat acagaaacgg tggaaagaac tttctaaaga agatttatct 960

cctatttgtg atcattcaca gaacagagtg atggttgaga agtacaatgg gaagagaata 1020

ggttcagaag atgaaaagtt aacagggctt ccttctaagg tgcaggatac tgtggaggag 1080

agcagtgaga gcattctacg gaaacgtatt cgagaggata gaaaggctac gacggctcag 1140

aaggtgcagc agatgaaaca gaggctaaat gaaactgaac gaaaaagaaa aaggccaaga 1200

ttgacagaca cctaaatgtt catgacttga gactattctg cagctataaa atttgaacct 1260

ttgatgtgca aagcaagacc tgaagcccac tccggaaact aaagtgaggc ttgctaaccc 1320

tgtagattgc ctcacaagtt gtctgtttac aaagtaagct ttccatccag gggatgaaga 1380

acgccaccag cagaagactt gcaaaccctt taatttgatg tattgttttt taacatgtgt 1440

atgaaatgta gaaagatgta aaggaaataa attaggagcg actactttgt attgtactgc 1500

cattcctaat gtatttttat actttttggc agcattaaat atttttatta aatag 1555

<210> SEQ ID NO 829
<211> LENGTH: 382
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 829

Met Ser Ala Thr Ile Glu Arg Glu Phe Glu Glu Leu Asp Ala Gln Cys

-continued

```
1               5                   10                  15

Arg Trp Gln Pro Leu Tyr Leu Glu Ile Arg Asn Glu Ser His Asp Tyr
            20                  25                  30

Pro His Arg Val Ala Lys Phe Pro Glu Asn Arg Asn Arg Asn Arg Tyr
        35                  40                  45

Arg Asp Val Ser Pro Tyr Asp His Ser Arg Val Lys Leu Gln Ser Thr
    50                  55                  60

Glu Asn Asp Tyr Ile Asn Ala Ser Leu Val Asp Ile Glu Glu Ala Gln
65                  70                  75                  80

Arg Ser Tyr Ile Leu Thr Gln Gly Pro Leu Pro Asn Thr Cys Cys His
                85                  90                  95

Phe Trp Leu Met Val Trp Gln Gln Lys Thr Lys Ala Val Val Met Leu
            100                 105                 110

Asn Arg Thr Val Glu Lys Glu Ser Val Lys Cys Ala Gln Tyr Trp Pro
        115                 120                 125

Thr Asp Asp Arg Glu Met Val Phe Lys Glu Thr Gly Phe Ser Val Lys
    130                 135                 140

Leu Leu Ser Glu Asp Val Lys Ser Tyr Tyr Thr Val His Leu Leu Gln
145                 150                 155                 160

Leu Glu Asn Ile Asn Thr Gly Glu Thr Arg Thr Ile Ser His Phe His
                165                 170                 175

Tyr Thr Thr Trp Pro Asp Phe Gly Val Pro Glu Ser Pro Ala Ser Phe
            180                 185                 190

Leu Asn Phe Leu Phe Lys Val Arg Glu Ser Gly Cys Leu Thr Pro Asp
        195                 200                 205

His Gly Pro Ala Val Ile His Cys Ser Ala Gly Ile Gly Arg Ser Gly
    210                 215                 220

Thr Phe Ser Leu Val Asp Thr Cys Leu Val Leu Met Glu Lys Gly Glu
225                 230                 235                 240

Asp Val Asn Val Lys Gln Leu Leu Leu Asn Met Arg Lys Tyr Arg Met
                245                 250                 255

Gly Leu Ile Gln Thr Pro Asp Gln Leu Arg Phe Ser Tyr Met Ala Ile
            260                 265                 270

Ile Glu Gly Ala Lys Tyr Thr Lys Gly Asp Ser Asn Ile Gln Lys Arg
        275                 280                 285

Trp Lys Glu Leu Ser Lys Glu Asp Leu Ser Pro Ile Cys Asp His Ser
    290                 295                 300

Gln Asn Arg Val Met Val Glu Lys Tyr Asn Gly Lys Arg Ile Gly Ser
305                 310                 315                 320

Glu Asp Glu Lys Leu Thr Gly Leu Pro Ser Lys Val Gln Asp Thr Val
                325                 330                 335

Glu Glu Ser Ser Glu Ser Ile Leu Arg Lys Arg Ile Arg Glu Asp Arg
            340                 345                 350

Lys Ala Thr Thr Ala Gln Lys Val Gln Gln Met Lys Gln Arg Leu Asn
        355                 360                 365

Glu Thr Glu Arg Lys Arg Lys Arg Pro Arg Leu Thr Asp Thr
    370                 375                 380
```

<210> SEQ ID NO 830
<211> LENGTH: 1666
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

-continued

<400> SEQUENCE: 830

```
ggccccccgt tccccgccag gctgcaggcg tcgggcctgg gccgtcaggg cagctgtgac      60
cggatcgctt cccgggcggc gagctggggg tgcacccgga ccgccgcccc cgggatcatg     120
ggcaatggca tgaccaaggt acttcctgga ctctacctcg gaaacttcat tgatgccaaa     180
gacctggatc agctgggccg aaataagatc acacacatca tctctatcca tgagtcaccc     240
cagcctctgc tgcaggatat cacctacctt cgcatcccgg tcgctgatac ccctgaggta     300
cccatcaaaa agcacttcaa agaatgtatc aacttcatcc actgctgccg ccttaatggg     360
gggaactgcc ttgtgcactg ctttgcaggc atctctcgca gcaccacgat tgtgacagcg     420
tatgtgatga ctgtgacggg gctaggctgg cgggacgtgc ttgaagccat caaggccacc     480
aggcccatcg ccaaccccaa cccaggcttt aggcagcagc ttgaagagtt tggctgggcc     540
agttcccaga agggtgccag acataggacc tcaaaaacct ctggtgccca atgccctccg     600
atgacttcag caacctggat ggtcaccgga cccaaagtac cagatctgtc tgtgcttcgg     660
tgaggaggac ccgggcccca cacagcaccc caaggagcag ctcatcatgg cggacgtgca     720
ggtgcagctt cggcctggga gctcgtcctg cactctaagt gcctcaaccg agcgcccaga     780
tgggtcctca accccctggca accccgatgg catcactcac cttcaatgca gctgcctcca     840
tcctaagcga gccgcttcct cttcttgtac ccgctgaagg cagcccccaa caggggggct     900
ccctactccc acccaaccct gcccacacta agcccataga cttggggcct cccccggcac     960
atcacccagg tctgccggac ggcagaggtg gatcgcggcc ttccactcct ctgtcacggg    1020
gccccggaac tcgagagtag gccacaccgc cccccagctg ggcatggggc ttcggcagga    1080
aactgaactt gatcttgagg ccccagaaag gcagcaactg gagcagaagc aagacttcat    1140
ctcttgctga cagcccaatt tgtcaatagc gctttcctca gagccagcct taacctgctg    1200
ttgagtccat taaaacgttt gcttaaagtt tttaccaata attagatcat cagggttgtt    1260
tagtgtggga tcaagccata acaaaactgc ctagcctctc aggggcctag aatttacaga    1320
accttcctcc tccctgcagc aagtctctct tctttattct gggggctggg aaggatccca    1380
aaacagggaa cttggccgaa ccctgggctt tggatgctaa ccactgaagt accagcacct    1440
gtaggatgct gtctttgaag aaactgaggc ggacctccaa atgcagccct aaggcagagg    1500
tcaacgtgga agaccagccc ttctccaagc cccactggtc tttgcaagct gtacgttgta    1560
ggcaatctga gaactggaaa gggggactac aaccagaaag ttggttaccc tgccatggga    1620
ataaagtagc tgttttccac cccaaaaaaa aaaaaaaaaa aaaaaa                  1666
```

<210> SEQ ID NO 831
<211> LENGTH: 181
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 831

```
Met Gly Asn Gly Met Thr Lys Val Leu Pro Gly Leu Tyr Leu Gly Asn
1               5                   10                  15

Phe Ile Asp Ala Lys Asp Leu Asp Gln Leu Gly Arg Asn Lys Ile Thr
            20                  25                  30

His Ile Ile Ser Ile His Glu Ser Pro Gln Pro Leu Leu Gln Asp Ile
        35                  40                  45
```

-continued

```
Thr Tyr Leu Arg Ile Pro Val Ala Asp Thr Pro Glu Val Pro Ile Lys
    50                  55                  60

Lys His Phe Lys Glu Cys Ile Asn Phe Ile His Cys Cys Arg Leu Asn
65                  70                  75                  80

Gly Gly Asn Cys Leu Val His Cys Phe Ala Gly Ile Ser Arg Ser Thr
                85                  90                  95

Thr Ile Val Thr Ala Tyr Val Met Thr Val Thr Gly Leu Gly Trp Arg
            100                 105                 110

Asp Val Leu Glu Ala Ile Lys Ala Thr Arg Pro Ile Ala Asn Pro Asn
        115                 120                 125

Pro Gly Phe Arg Gln Gln Leu Glu Glu Phe Gly Trp Ala Ser Ser Gln
    130                 135                 140

Lys Gly Ala Arg His Arg Thr Ser Lys Thr Ser Gly Ala Gln Cys Pro
145                 150                 155                 160

Pro Met Thr Ser Ala Thr Trp Met Val Thr Gly Pro Lys Val Pro Asp
                165                 170                 175

Leu Ser Val Leu Arg
            180
```

<210> SEQ ID NO 832
<211> LENGTH: 1807
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 832

```
ggccccccgt tccccgccag gctgcaggcg tcgggcctgg gccgtcaggg cagctgtgac      60

cggatcgctt cccgggcggc gagctggggg tgcacccgga ccgccgcccc cgggatcatg     120

ggcaatggca tgaccaaggt acttcctgga ctctacctcg gaaacttcat tgatgccaaa     180

gacctggatc agctgggccg aaataagatc acacacatca tctctatcca tgagtcaccc     240

cagcctctgc tgcaggatat cacctacctt cgcatcccgg tcgctgatac ccctgaggta     300

cccatcaaaa agcacttcaa agaatgtatc aacttcatcc actgctgccg ccttaatggg     360

gggaactgcc ttgtgcactg ctttgcaggc atctctcgca gcaccacgat tgtgacagcg     420

tatgtgatga ctgtgacggg gctaggctgg cgggacgtgc ttgaagccat caaggccacc     480

aggcccatcg ccaaccccaa cccaggcttt aggcagcagc ttgaagagtt tggctgggcc     540

agttcccaga agggtgccag acataggacc tcaaaaacct ctggtgccca atgccctccg     600

atgacttcag caacctgcct gctggctgca cgtgtggctc ttctctccgc agcgctggtg     660

cgcgaagcca ccgggcgcac agcccagcgc tgtcgtctga gtccgcgggc ggccgccgag     720

cgcctgctgg ggccgccacc tcacgttgca gcaggatggt caccggaccc aaagtaccag     780

atctgtctgt gcttcggtga ggaggacccg ggccccacac agcaccccaa ggagcagctc     840

atcatggcgg acgtgcaggt gcagcttcgg cctgggagct cgtcctgcac tctaagtgcc     900

tcaaccgagc gcccagatgg gtcctcaacc cctggcaacc ccgatggcat cactcacctt     960

caatgcagct gcctccatcc taagcgagcc gcttcctctt cttgtacccg ctgaaggcag    1020

cccccaacag gggggctccc tactcccacc caaccctgcc cacactaagc ccatagactt    1080

ggggcctccc cggcggcaca tcacccaggt ctgccggacg gcagaggtgg atcgcggcct    1140

tccactcctc tgtcacgggg ccccggaact cgagagtagg ccacaccgcc ccccagctgg    1200

gcatggggct tcggcaggaa actgaacttg atcttgaggc cccagaaagg cagcaactgg    1260
```

-continued

agcagaagca agacttcatc tcttgctgac agcccaattt gtcaatagcg ctttcctcag 1320

agccagcctt aacctgctgt tgagtccatt aaaacgtttg cttaaagttt ttaccaataa 1380

ttagatcatc agggttgttt agtgtgggat caagccataa caaaactgcc tagcctctca 1440

ggggcctaga atttacagaa ccttcctcct ccctgcagct agtctctctt ctttattctg 1500

ggggctggga aggatcccaa aacagggaac ttggccgaac cctgggcttt ggatgctaac 1560

cactgaagta ccagcacctg taggatgctg tctttgaaga aactgaggcg gacctccaaa 1620

tgcagcccta aggcagaggt caacgtggaa gaccagccct tctccaagcc ccactggtct 1680

ttgcaagctg tacgttgtag gcaatctgag aactggaaag ggggactaca accagaaagt 1740

tggttaccct gccatgggaa taaagtagct gttttccacc ccaaaaaaaa aaaaaaaaaa 1800

aaaaaaa 1807

<210> SEQ ID NO 833
<211> LENGTH: 298
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 833

Met Gly Asn Gly Met Thr Lys Val Leu Pro Gly Leu Tyr Leu Gly Asn
1 5 10 15

Phe Ile Asp Ala Lys Asp Leu Asp Gln Leu Gly Arg Asn Lys Ile Thr
20 25 30

His Ile Ile Ser Ile His Glu Ser Pro Gln Pro Leu Leu Gln Asp Ile
35 40 45

Thr Tyr Leu Arg Ile Pro Val Ala Asp Thr Pro Glu Val Pro Ile Lys
50 55 60

Lys His Phe Lys Glu Cys Ile Asn Phe Ile His Cys Cys Arg Leu Asn
65 70 75 80

Gly Gly Asn Cys Leu Val His Cys Phe Ala Gly Ile Ser Arg Ser Thr
85 90 95

Thr Ile Val Thr Ala Tyr Val Met Thr Val Thr Gly Leu Gly Trp Arg
100 105 110

Asp Val Leu Glu Ala Ile Lys Ala Thr Arg Pro Ile Ala Asn Pro Asn
115 120 125

Pro Gly Phe Arg Gln Gln Leu Glu Glu Phe Gly Trp Ala Ser Ser Gln
130 135 140

Lys Gly Ala Arg His Arg Thr Ser Lys Thr Ser Gly Ala Gln Cys Pro
145 150 155 160

Pro Met Thr Ser Ala Thr Cys Leu Leu Ala Ala Arg Val Ala Leu Leu
165 170 175

Ser Ala Ala Leu Val Arg Glu Ala Thr Gly Arg Thr Ala Gln Arg Cys
180 185 190

Arg Leu Ser Pro Arg Ala Ala Ala Glu Arg Leu Leu Gly Pro Pro Pro
195 200 205

His Val Ala Ala Gly Trp Ser Pro Asp Pro Lys Tyr Gln Ile Cys Leu
210 215 220

Cys Phe Gly Glu Glu Asp Pro Gly Pro Thr Gln His Pro Lys Glu Gln
225 230 235 240

Leu Ile Met Ala Asp Val Gln Val Gln Leu Arg Pro Gly Ser Ser Ser
245 250 255

-continued

```
Cys Thr Leu Ser Ala Ser Thr Glu Arg Pro Asp Gly Ser Ser Thr Pro
            260                 265                 270

Gly Asn Pro Asp Gly Ile Thr His Leu Gln Cys Ser Cys Leu His Pro
        275                 280                 285

Lys Arg Ala Ala Ser Ser Ser Cys Thr Arg
    290                 295
```

<210> SEQ ID NO 834
<211> LENGTH: 1268
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 834

```
ggccccccgt tccccgccag gctgcaggcg tcgggcctgg gccgtcaggg cagctgtgac     60

cggatcgctt cccgggcggc gagctggggg tgcacccgga ccgccgcccc cgggatcatg    120

ggcaatggca tgaccaaggt acttcctgga ctctacctcg gaaacttcat tgatgccaaa    180

gacctggatc agctgggccg aaataagatc acacacatca tctctatcca tgagtcaccc    240

cagcctctgc tgcaggatat cacctacctt cgcatcccgg tcgctgatac ccctgaggta    300

cccatcaaaa agcacttcaa agaatgtatc aacttcatcc actgctgccg ccttaatggg    360

gggaactgcc ttgtgcactg ctttgcaggc atctctcgca gcaccacgat tgtgacagcg    420

tatgtgatga ctgtgacggg gctaggctgg cgggacgtgc ttgaagccat caaggccacc    480

aggcccatcg ccaaccccaa cccaggcttt aggcagcagc ttgaagagtt tggctgggcc    540

agttcccaga agggtgccag acataggacc tcaaaaacct ctggtgccca atgccctccg    600

atgacttcag caacctggat ggtcaccgga cccaaagtac cagatctgtc tgtgcttcgg    660

tgaggaggac ccgggcccca cacagcaccc caaggagcag ctcatcatgg cggacgtgca    720

ggtgcagctt cggcctggga gctcgtcctg cactctaagt gcctcaaccg agcgcccaga    780

tgggtcctca acccctggca accccgatgg catcactcac cttcaatgca gcttgcctcc    840

atcctaagcg agccgcttcc tcttcttgta cccgctgaag gcaagccccc aacagggggg    900

ctccctactc ccacccaacc ctgcccacac taagcccata gacttggggc ctccccggc     960

acatcaccca ggtctgccgg acggcagagg tggatcgcgg ccttccactc ctctgtcacg   1020

gggccccgga actcgagagt aggcctcacc gccccccagc tgggcatggg gcttcggcag   1080

gaaactgaac ttgatcttga ggccagcaga aaggcagcaa ctggagcaga agcaagactt   1140

catctcttgc tgacagccca atttgtcaat agcgctttcc tcagagccag ccttaacctg   1200

ctgttgagtc cattaaaacg tttgcttaaa gtttttacca ataaaaaaaa aaaaaaaaaa   1260

aaaaaaaa                                                            1268
```

<210> SEQ ID NO 835
<211> LENGTH: 181
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 835

```
Met Gly Asn Gly Met Thr Lys Val Leu Pro Gly Leu Tyr Leu Gly Asn
1               5                   10                  15

Phe Ile Asp Ala Lys Asp Leu Asp Gln Leu Gly Arg Asn Lys Ile Thr
            20                  25                  30
```

-continued

```
His Ile Ile Ser Ile His Glu Ser Pro Gln Pro Leu Leu Gln Asp Ile
        35                  40                  45

Thr Tyr Leu Arg Ile Pro Val Ala Asp Thr Pro Glu Val Pro Ile Lys
    50                  55                  60

Lys His Phe Lys Glu Cys Ile Asn Phe Ile His Cys Cys Arg Leu Asn
65                  70                  75                  80

Gly Gly Asn Cys Leu Val His Cys Phe Ala Gly Ile Ser Arg Ser Thr
                85                  90                  95

Thr Ile Val Thr Ala Tyr Val Met Thr Val Thr Gly Leu Gly Trp Arg
            100                 105                 110

Asp Val Leu Glu Ala Ile Lys Ala Thr Arg Pro Ile Ala Asn Pro Asn
        115                 120                 125

Pro Gly Phe Arg Gln Gln Leu Glu Glu Phe Gly Trp Ala Ser Ser Gln
    130                 135                 140

Lys Gly Ala Arg His Arg Thr Ser Lys Thr Ser Gly Ala Gln Cys Pro
145                 150                 155                 160

Pro Met Thr Ser Ala Thr Trp Met Val Thr Gly Pro Lys Val Pro Asp
                165                 170                 175

Leu Ser Val Leu Arg
            180
```

<210> SEQ ID NO 836
<211> LENGTH: 1045
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 836

```
ggcccccgt tccccgccag gctgcaggcg tcgggcctgg gccgtcaggg cagctgtgac      60

cggatcgctt cccgggcggc gagctggggg tgcacccgga ccgccgcccc cgggatcatg    120

ggcaatggca tgaccaaggt acttcctgga ctctacctcg gaaacttcat tgatgccaaa    180

gacctggatc agctgggccg aaataagatc acacacatca tctctatcca tgagtcaccc    240

cagcctctgc tgcaggatat cacctacctt cgcatcccgg tcgctgatac ccctgaggta    300

cccatcaaaa agcacttcaa agaatgtatc aacttcatcc actgctgccg ccttaatggg    360

gggaactgcc ttgtgcactg ctttgcaggc atctctcgca gcaccacgat tgtgacagcg    420

tatgtgatga ctgtgacggg gctaggctgg cgggacgtgc ttgaagccat caaggccacc    480

aggcccatcg ccaaccccaa cccaggcttt aggcagcagc ttgaagagtt tggctgggcc    540

agttcccaga agggtgccag acataggacc tcaaaaacct ctggtgccca atgccctccg    600

atgacttcag caacctggat ggtcaccgga cccaaagtac cagatctgtc tgtgcttcgg    660

tgaggaggac ccgggcccca cacagcaccc caaggagcag ctcatcatgg cggacctagt    720

ctctcttctt tattctgggg gctgggaagg atcccaaaac agggaacttg gccgaaccct    780

gggctttgga tgctaaccac tgaagtacca gcacctgtag gatgctgtct ttgaagaaac    840

tgaggcggac ctccaaatgc agccctaagg cagaggtcaa cgtggaagac cagcccttct    900

ccaagcccca ctggtctttg caagctgtac gttgtaggca atctgagaac tggaaagggg    960

gactacaacc agaaagttgg ttaccctgcc atgggaataa agtagctgtt ttccacccca   1020

taaaaaaaaa aaaaaaaaaa aaaaa                                          1045
```

<210> SEQ ID NO 837

-continued

<211> LENGTH: 181
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 837

Met Gly Asn Gly Met Thr Lys Val Leu Pro Gly Leu Tyr Leu Gly Asn
1   5   10   15

Phe Ile Asp Ala Lys Asp Leu Asp Gln Leu Gly Arg Asn Lys Ile Thr
            20   25   30

His Ile Ile Ser Ile His Glu Ser Pro Gln Pro Leu Leu Gln Asp Ile
        35   40   45

Thr Tyr Leu Arg Ile Pro Val Ala Asp Thr Pro Glu Val Pro Ile Lys
    50   55   60

Lys His Phe Lys Glu Cys Ile Asn Phe Ile His Cys Cys Arg Leu Asn
65   70   75   80

Gly Gly Asn Cys Leu Val His Cys Phe Ala Gly Ile Ser Arg Ser Thr
                85   90   95

Thr Ile Val Thr Ala Tyr Val Met Thr Val Thr Gly Leu Gly Trp Arg
            100   105   110

Asp Val Leu Glu Ala Ile Lys Ala Thr Arg Pro Ile Ala Asn Pro Asn
        115   120   125

Pro Gly Phe Arg Gln Gln Leu Glu Glu Phe Gly Trp Ala Ser Ser Gln
    130   135   140

Lys Gly Ala Arg His Arg Thr Ser Lys Thr Ser Gly Ala Gln Cys Pro
145   150   155   160

Pro Met Thr Ser Ala Thr Trp Met Val Thr Gly Pro Lys Val Pro Asp
                165   170   175

Leu Ser Val Leu Arg
            180

<210> SEQ ID NO 838
<211> LENGTH: 982
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 838

ggccccccgt tccccgccag gctgcaggcg tcgggcctgg gccgtcaggg cagctgtgac 60

cggatcgctt cccgggcggc gagctggggg tgcacccgga ccgccgcccc cgggatcatg 120

ggcaatggca tgaccaaggt acttcctgga ctctacctcg gaaacttcat tgatgccaaa 180

gacctggatc agctgggccg aaataagatc acacacatca tctctatcca tgagtcaccc 240

cagcctctgc tgcaggatat cacctacctt cgcatcccgg tcgctgatac ccctgaggta 300

cccatcaaaa agcacttcaa agaatgtatc aacttcatcc actgctgccg ccttaatggg 360

gggaactgcc ttgtgcactg ctttgcaggc atctctcgca gcaccacgat tgtgacagcg 420

tatgtgatga ctgtgacggg gctaggctgg cgggacgtgc ttgaagccat caaggccacc 480

aggcccatcg ccaaccccaa cccaggcttt aggcagcagc ttaagagttt ggctgggcca 540

gttcccagaa ggatggtcac cggacccaaa gtaccagatc tgtctgtgct tcggtgagga 600

ggacccgggc cccacacagc accccaagga gcagctcatc atggcggacc tagtctctct 660

tctttattct gggggctggg aaggatccca aaacagggaa cttggccgaa ccctgggctt 720

tggatgctaa ccactgaagt accagcacct gtaggatgct gtctttgaag aaactgaggc 780

-continued

```
ggacctccaa atgcagccct aaggcagagg tcaacgtgga agaccagccc ttctccaagc    840

cccactggtc tttgcaagct gtacgttgta ggcaatctga gaactggaaa gggggactac    900

aaccagaaag ttggttaccc tgccatggga ataaagtagc tgttttccac cccccaaaaa    960

aaaaaaaaaa aaaaaaaaaa aa                                             982
```

```
<210> SEQ ID NO 839
<211> LENGTH: 159
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 839

Met Gly Asn Gly Met Thr Lys Val Leu Pro Gly Leu Tyr Leu Gly Asn
1               5                   10                  15

Phe Ile Asp Ala Lys Asp Leu Asp Gln Leu Gly Arg Asn Lys Ile Thr
            20                  25                  30

His Ile Ile Ser Ile His Glu Ser Pro Gln Pro Leu Leu Gln Asp Ile
        35                  40                  45

Thr Tyr Leu Arg Ile Pro Val Ala Asp Thr Pro Glu Val Pro Ile Lys
    50                  55                  60

Lys His Phe Lys Glu Cys Ile Asn Phe Ile His Cys Cys Arg Leu Asn
65                  70                  75                  80

Gly Gly Asn Cys Leu Val His Cys Phe Ala Gly Ile Ser Arg Ser Thr
                85                  90                  95

Thr Ile Val Thr Ala Tyr Val Met Thr Val Thr Gly Leu Gly Trp Arg
            100                 105                 110

Asp Val Leu Glu Ala Ile Lys Ala Thr Arg Pro Ile Ala Asn Pro Asn
        115                 120                 125

Pro Gly Phe Arg Gln Gln Leu Lys Ser Leu Ala Gly Pro Val Pro Arg
    130                 135                 140

Arg Met Val Thr Gly Pro Lys Val Pro Asp Leu Ser Val Leu Arg
145                 150                 155
```

```
<210> SEQ ID NO 840
<211> LENGTH: 1064
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 840

ggccccccgt tccccgccag gctgcaggcg tcgggcctgg gccgtcaggg cagctgtgac     60

cggatcgctt cccgggcggc gagctggggg tgcacccgga ccgccgcccc cgggatcatg    120

ggcaatggca tgaccaaggt acttcctgga ctctacctcg gaaacttcat tgatgccaaa    180

gacctggatc agctgggccg aaataagatc acacacatca tctctatcca tgagtcaccc    240

cagcctctgc tgcaggatat cacctacctt cgcatcccgg tcgctgatac ccctgaggta    300

cccatcaaaa agcacttcaa agaatgtatc aacttcatcc actgctgccg ccttaatggg    360

gggaactgcc ttgtgcactg ctttgcaggc atctctcgca gcaccacgat tgtgacagcg    420

tatgtgatga ctgtgacggg gctaggctgg cgggacgtgc ttgaagccat caaggccacc    480

aggcccatcg ccaaccccaa cccaggcttt aggcagcagc ttgaagagtt tggctgggcc    540

agttcccaga agggctttta ccaacctcat aagctgttgt gagaaccaat tgagacactg    600

caggaaagtg tttagccagg cccagcactg atgagcagtc ggatggtcac cggacccaaa    660
```

-continued

```
gtaccagatc tgtctgtgct tcggtgagga ggacccgggc cccacacagc accccaagga    720

gcagctcatc atggcggacc tagtctctct tctttattct gggggctggg aaggatccca    780

aaacagggaa cttggccgaa ccctgggctt tggatgctaa ccactgaagt accagcacct    840

gtaggatgct gtctttgaag aaactgaggc ggacctccaa atgcagccct aaggcagagg    900

tcaacgtgga agaccagccc ttctccaagc cccactggtc tttgcaagct gtacgttgta    960

ggcaatctga gaactggaaa gggggactac aaccagaaag ttggttaccc tgccatggga   1020

ataaagtagc tgttttccaa aaaaaaaaaa aaaaaaaaaa aaaa                    1064


<210> SEQ ID NO 841
<211> LENGTH: 154
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 841

Met Gly Asn Gly Met Thr Lys Val Leu Pro Gly Leu Tyr Leu Gly Asn
1               5                   10                  15

Phe Ile Asp Ala Lys Asp Leu Asp Gln Leu Gly Arg Asn Lys Ile Thr
            20                  25                  30

His Ile Ile Ser Ile His Glu Ser Pro Gln Pro Leu Leu Gln Asp Ile
        35                  40                  45

Thr Tyr Leu Arg Ile Pro Val Ala Asp Thr Pro Glu Val Pro Ile Lys
    50                  55                  60

Lys His Phe Lys Glu Cys Ile Asn Phe Ile His Cys Cys Arg Leu Asn
65                  70                  75                  80

Gly Gly Asn Cys Leu Val His Cys Phe Ala Gly Ile Ser Arg Ser Thr
                85                  90                  95

Thr Ile Val Thr Ala Tyr Val Met Thr Val Thr Gly Leu Gly Trp Arg
            100                 105                 110

Asp Val Leu Glu Ala Ile Lys Ala Thr Arg Pro Ile Ala Asn Pro Asn
        115                 120                 125

Pro Gly Phe Arg Gln Gln Leu Glu Glu Phe Gly Trp Ala Ser Ser Gln
    130                 135                 140

Lys Gly Phe Tyr Gln Pro His Lys Leu Leu
145                 150


<210> SEQ ID NO 842
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Small interfering RNA - PRL-3

<400> SEQUENCE: 842

agacccggug cugcguuau                                                  19
```

What is claimed is:

1. An isolated small interfering RNA (siRNA) polynucleotide, comprising at least one nucleotide sequence selected from the group consisting of SEQ ID NOS:4-7, 100-103, 105-108, 120-123, 125-128, 130-133, 140-143, 145-148, 150-153, 440-443, 445-448, 455-458, 460-463, 465-468, 470-473, 475-478, 480-483, 485-488, and 490-493.

2. The small interfering RNA polynucleotide of claim 1 that comprises at least one nucleotide sequence selected from the group consisting of SEQ ID NOS: 4-7, 100-103, 105-108, 120-123, 125-128, 130-133, 140-143, 145-148, 150-153, 440-443, 445-448, 455-458, 460-463, 465-468, 470-473, 475-478, 480-483, 485-488, and 490-493 and the complementary polynucleotide thereto.

3. A small interfering RNA polynucleotide of either claim 1 or claim 2 that is capable of interfering with expression of a polypeptide, which polypeptide comprises an amino acid sequence as set forth in a sequence selected from the group

consisting of SEQ ID NO: 779, SEQ ID NO:789, SEQ ID NO:791, SEQ ID NO:797, SEQ ID NO:799, SEQ ID NO:801, SEQ ID NO:803, SEQ ID NO:805, SEQ ID NO:807, SEQ ID NO:809, SEQ ID NO:811, and SEQ ID NO:813.

4. The siRNA polynucleotide of either claim 1 or claim 2 wherein the nucleotide sequence of the siRNA polynucleotide differs by one, two, three or four nucleotides at any of positions 1-19 of a sequence selected from the group consisting of the sequences set forth in SEQ ID NOS: 4-7, 100-103, 105-108, 120-123, 125-128, 130-133, 140-143, 145-148, 150-153, 440-443, 445-448, 455-458, 460-463, 465-468, 470-473, 475-478, 480-483, 485-488, and 490-493.

5. The siRNA polynucleotide of either claim 1 or claim 2 wherein the nucleotide sequence of the siRNA polynucleotide differs by at least two, three or four nucleotides at any of positions 1-19 of a sequence selected from the group consisting of the sequences set forth in SEQ ID NOS: 4-7, 100-103, 105-108, 120-123, 125-128, 130-133, 140-143, 145-148, 150-153, 440-443, 445-448, 455-458, 460-463, 465-468, 470-473, 475-478, 480-483, 485-488, and 490-493.

6. An isolated siRNA polynucleotide comprising a nucleotide sequence according to SEQ ID NO: 4, or the complement thereof.

7. An isolated siRNA polynucleotide comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS: 100 and 105, or the complement thereof.

8. An isolated siRNA polynucleotide comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS: 120, 125, and 130, or the complement thereof.

9. An isolated siRNA polynucleotide comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS: 140, 145, and 150, or the complement thereof.

10. An isolated siRNA polynucleotide comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS: 440 and 445, or the complement thereof.

11. An isolated siRNA polynucleotide comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS: 455 and 460, or the complement thereof.

12. An isolated siRNA polynucleotide comprising a nucleotide sequence according to SEQ ID NO: 465, or the complement thereof.

13. An isolated siRNA polynucleotide comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS: 470 and 475, or the complement thereof.

14. An isolated siRNA polynucleotide comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS: 480, 485, and 490, or the complement thereof.

15. The siRNA polynucleotide of claim 1 or claim 2 wherein the polynucleotide comprises at least one synthetic nucleotide analogue of a naturally occurring nucleotide.

16. The siRNA polynucleotide of claim 1 or claim 2 wherein the polynucleotide is linked to a detectable label.

17. The siRNA polynucleotide of claim 16 wherein the detectable label is a reporter molecule.

18. The siRNA of claim 17 wherein the reporter molecule is selected from the group consisting of a dye, a radionuclide, a luminescent group, a fluorescent group, and biotin.

19. The siRNA polynucleotide of claim 18 wherein the fluorescent group is fluorescein isothiocyanate.

20. The siRNA polynucleotide of claim 16 wherein the detectable label is a magnetic particle.

21. A pharmaceutical composition comprising the siRNA polynucleotide of either claim 1 or claim 2 and a physiologically acceptable carrier.

22. The pharmaceutical composition of claim 22 wherein the carrier comprises a liposome.

23. A recombinant nucleic acid construct comprising a polynucleotide that is capable of directing transcription of a small interfering RNA (siRNA), the polynucleotide comprising:

(i) a first promoter; (ii) a second promoter; and (iii) at least one DNA polynucleotide segment comprising at least one nucleotide sequence selected from the group consisting of SEQ ID NOS: 4-7, 100-103, 105-108, 120-123, 125-128, 130-133, 140-143, 145-148, 150-153, 440-443, 445-448, 455-458, 460-463, 465-468, 470-473, 475-478, 480-483, 485-488, and 490-493, or a complement thereto, wherein each DNA polynucleotide segment and its complement are operably linked to at least one of the first and second promoters, and wherein the promoters are oriented to direct transcription of the DNA polynucleotide segment and its reverse complement.

24. The recombinant nucleic acid construct of claim 23, comprising at least one enhancer that is selected from a first enhancer operably linked to the first promoter and a second enhancer operably linked to the second promoter.

25. The recombinant nucleic acid construct of claim 23, comprising at least one transcriptional terminator that is selected from (i) a first transcriptional terminator that is positioned in the construct to terminate transcription directed by the first promoter and (ii) a second transcriptional terminator that is positioned in the construct to terminate transcription directed by the second promoter.

26. The recombinant nucleic acid construct of claim 24 wherein the siRNA is capable of interfering with expression of a polypeptide, wherein the polypeptide comprises an amino acid sequence as set forth in a sequence selected from the group consisting of SEQ ID NO: 779, SEQ ID NO:789, SEQ ID NO:791, SEQ ID NO:797, SEQ ID NO:799, SEQ ID NO:801, SEQ ID NO:803, SEQ ID NO:805, SEQ ID NO:807, SEQ ID NO:809, SEQ ID NO:811, and SEQ ID NO:813.

27. A recombinant nucleic acid construct comprising a polynucleotide that is capable of directing transcription of a small interfering RNA (siRNA), the polynucleotide comprising at least one promoter and a DNA polynucleotide segment, wherein the DNA polynucleotide segment is operably linked to the promoter, and wherein the DNA polynucleotide segment comprises (i) at least one DNA polynucleotide that comprises at least one nucleotide sequence selected from the group consisting of SEQ ID NOS: 4-7, 100-103, 105-108, 120-123, 125-128, 130-133, 140-143, 145-148, 150-153, 440-443, 445-448, 455-458, 460-463, 465-468, 470-473, 475-478, 480-483, 485-488, and 490-493, or a complement thereto; (ii) a spacer sequence comprising at least 4 nucleotides operably linked to the DNA polynucleotide of (i); and (iii) the reverse complement of the DNA polynucleotide of (i) operably linked to the spacer sequence.

28. The recombinant nucleic acid construct of claim 27 wherein the siRNA comprises an overhang of at least one and no more than four nucleotides, the overhang being located immediately 3' to (iii).

29. The recombinant nucleic acid construct of claim 27 wherein the spacer sequence comprises at least 9 nucleotides.

30. The recombinant nucleic acid construct of claim 27 wherein the spacer sequence comprises two uridine nucleotides that are contiguous with (iii).

31. The recombinant nucleic acid construct of claim 27 comprising at least one transcriptional terminator that is operably linked to the DNA polynucleotide segment.

32. A host cell transformed or transfected with the recombinant nucleic acid construct of any one of claims **23**-**31**.

33. A pharmaceutical composition comprising an siRNA polynucleotide and a physiologically acceptable carrier, wherein the siRNA polynucleotide is selected from the group consisting of:

(i) an RNA polynucleotide which comprises at least one nucleotide sequence selected from the group consisting of SEQ ID NOS: 4-7, 100-103, 105-108, 120-123, 125-128, 130-133, 140-143, 145-148, 150-153, 440-443, 445-448, 455-458, 460-463, 465-468, 470-473, 475-478, 480-483, 485-488, and 490-493,

(ii) an RNA polynucleotide that comprises at least one nucleotide sequence selected from the group consisting of SEQ ID NOS: 4-7, 100-103, 105-108, 120-123, 125-128, 130-133, 140-143, 145-148, 150-153, 440-443, 445-448, 455-458, 460-463, 465-468, 470-473, 475-478, 480-483, 485-488, and 490-493 and the complementary polynucleotide thereto,

(iii) an RNA polynucleotide according to (i) or (ii) wherein the nucleotide sequence of the siRNA polynucleotide differs by one, two or three nucleotides at any of positions 1-19 of a sequence selected from the group consisting of the sequences set forth in SEQ ID NOS: 4-7, 100-103, 105-108, 120-123, 125-128, 130-133, 140-143, 145-148, 150-153, 440-443, 445-448, 455-458, 460-463, 465-468, 470-473, 475-478, 480-483, 485-488, and 490-493, and

(iv) an RNA polynucleotide according to (i) or (ii) wherein the nucleotide sequence of the siRNA polynucleotide differs by two, three or four nucleotides at any of positions 1-19 of a sequence selected from the group consisting of the sequences set forth in SEQ ID NOS: 4-7, 100-103, 105-108, 120-123, 125-128, 130-133, 140-143, 145-148, 150-153, 440-443, 445-448, 455-458, 460-463, 465-468, 470-473, 475-478, 480-483, 485-488, and 490-493.

34. The pharmaceutical composition of claim 33 wherein the carrier comprises a liposome.

35. A method for interfering with expression of a polypeptide, or variant thereof, comprising contacting a subject that comprises at least one cell which is capable of expressing the polypeptide with a siRNA polynucleotide for a time and under conditions sufficient to interfere with expression of the polypeptide, wherein:

(a) the polypeptide comprises an amino acid sequence as set forth in a sequence selected from the group consisting of SEQ ID NO: 779, SEQ ID NO:789, SEQ ID NO:791, SEQ ID NO:797, SEQ ID NO:799, SEQ ID NO:801, SEQ ID NO:803, SEQ ID NO:805, SEQ ID NO:807, SEQ ID NO:809, SEQ ID NO:811, and SEQ ID NO:813,

(b) the siRNA polynucleotide is selected from the group consisting of

(i) an RNA polynucleotide which comprises at least one nucleotide sequence selected from the group consisting of SEQ ID NOS: 4-7, 100-103, 105-108, 120-123, 125-128, 130-133, 140-143, 145-148, 150-153, 440-443, 445-448, 455-458, 460-463, 465-468, 470-473, 475-478, 480-483, 485-488, and 490-493,

(ii) an RNA polynucleotide that comprises at least one nucleotide sequence selected from the group consisting of SEQ ID NOS: 4-7, 100-103, 105-108, 120-123, 125-128, 130-133, 140-143, 145-148, 150-153, 440-443, 445-448, 455-458, 460-463, 465-468, 470-473, 475-478, 480-483, 485-488, and 490-493 and the complementary polynucleotide thereto,

(iii) an RNA polynucleotide according to (i) or (ii) wherein the nucleotide sequence of the siRNA polynucleotide differs by one, two or three nucleotides at any of positions 1-19 of a sequence selected from the group consisting of the sequences set forth in SEQ ID NOS: 4-7, 100-103, 105-108, 120-123, 125-128, 130-133, 140-143, 145-148, 150-153, 440-443, 445-448, 455-458, 460-463, 465-468, 470-473, 475-478, 480-483, 485-488, and 490-493, and

(iv) an RNA polynucleotide according to (i) or (ii) wherein the nucleotide sequence of the siRNA polynucleotide differs by two, three or four nucleotides at any of positions 1-19 of a sequence selected from the group consisting of the sequences set forth in SEQ ID NOS: 4-7, 100-103, 105-108, 120-123, 125-128, 130-133, 140-143, 145-148, 150-153, 440-443, 445-448, 455-458, 460-463, 465-468, 470-473, 475-478, 480-483, 485-488, and 490-493.

36. A method for interfering with expression of a polypeptide that comprises an amino acid sequence as set forth in a sequence selected from the group consisting of SEQ ID NO: 779, SEQ ID NO:789, SEQ ID NO:791, SEQ ID NO:797, SEQ ID NO:799, SEQ ID NO:801, SEQ ID NO:803, SEQ ID NO:805, SEQ ID NO:807, SEQ ID NO:809, SEQ ID NO:811, and SEQ ID NO:813, or a variant of said polypeptide, said method comprising contacting, under conditions and for a time sufficient to interfere with expression of the polypeptide, (i) a subject that comprises at least one cell that is capable of expressing the polypeptide, and (ii) a recombinant nucleic acid construct according to either claim 23 or claim 27.

37. A method for identifying a component of a signal transduction pathway comprising:

A. contacting a siRNA polynucleotide and a first biological sample comprising at least one cell that is capable of expressing a target polypeptide, or a variant of said polypeptide, under conditions and for a time sufficient for target polypeptide expression when the siRNA polynucleotide is not present, wherein

(1) the target polypeptide comprises an amino acid sequence as set forth in a sequence selected from the group consisting of SEQ ID NO: 779, SEQ ID NO: 789, SEQ ID NO: 791, SEQ ID NO: 797, SEQ ID NO: 799, SEQ ID NO: 801, SEQ ID NO: 803, SEQ ID NO: 805, SEQ ID NO: 807, SEQ ID NO: 809, SEQ ID NO: 811, and SEQ ID NO: 813,

(2) the siRNA polynucleotide is selected from the group consisting of

(i) an RNA polynucleotide which comprises at least one nucleotide sequence selected from the group consisting of SEQ ID NOS: 4-7, 100-103, 105-108, 120-123, 125-128, 130-133, 140-143, 145-148, 150-153, 440-443, 445-448, 455-458, 460-463, 465-468, 470-473, 475-478, 480-483, 485-488, and 490-493,

(ii) an RNA polynucleotide that comprises at least one nucleotide sequence selected from the group consisting of SEQ ID NOS: 4-7, 100-103, 105-108, 120-123, 125-128, 130-133, 140-143, 145-148, 150-153, 440-443, 445-448, 455-458, 460-463, 465-468, 470-473, 475-478, 480-483, 485-488, and 490-493 and the complementary polynucleotide thereto,

(iii) an RNA polynucleotide according to (i) or (ii) wherein the nucleotide sequence of the siRNA polynucleotide differs by one, two or three nucleotides at any of positions 1-19 of a sequence selected from the group consisting of the sequences set forth in SEQ ID NOS: 4-7, 100-103, 105-108, 120-123, 125-128, 130-133, 140-143, 145-148, 150-153, 440-443, 445-448, 455-458, 460-463, 465-468, 470-473, 475-478, 480-483, 485-488, and 490-493, and

(iv) an RNA polynucleotide according to (i) or (ii) wherein the nucleotide sequence of the siRNA polynucleotide differs by two, three or four nucleotides at any of positions 1-19 of a sequence selected from the group consisting of the sequences set forth in SEQ ID NOS: 4-7, 100-103, 105-108, 120-123, 125-128, 130-133, 140-143, 145-148, 150-153, 440-443, 445-448, 455-458, 460-463, 465-468, 470-473, 475-478, 480-483, 485-488, and 490-493; and

B. comparing a level of phosphorylation of at least one protein that is capable of being phosphorylated in the cell with a level of phosphorylation of the protein in a control sample that has not been contacted with the siRNA polynucleotide,

wherein an altered level of phosphorylation of the protein in the presence of the siRNA polynucleotide relative to the level of phosphorylation of the protein in an absence of the siRNA polynucleotide indicates that the protein is a component of a signal transduction pathway.

* * * * *

US 20030157573A1

(19) **United States**

(12) **Patent Application Publication** (10) **Pub. No.: US 2003/0157573 A1**

Mor (43) **Pub. Date: Aug. 21, 2003**

(54) **USE OF AXL RECEPTOR FOR DIAGNOSIS AND TREATMENT OF RENAL DISEASE**

(76) Inventor: **Orna Mor**, Kiryat Ono (IL)

Correspondence Address:
John P. White
Cooper & Dunham LLP
1185 Avenue of the Americas
New York, NY 10036 (US)

(21) Appl. No.: **10/365,135**

(22) Filed: **Feb. 12, 2003**

Related U.S. Application Data

(60) Provisional application No. 60/356,374, filed on Feb. 12, 2002.

Publication Classification

(51) **Int. Cl.^{7}** **A61K 31/00**; G01N 33/53; G01N 33/567

(52) **U.S. Cl.** **435/7.2**; 514/1

(57) **ABSTRACT**

The invention is directed to a process of identifying a compound capable of inhibiting the activity of a human Ax1 receptor that comprises contacting the Ax1 receptor or cells expressing the Ax1 receptor with the compound; measuring the Ax1 receptor activity in the presence of the compound ; and comparing the activity measured to that measured in the absence of the compound under controlled conditions, wherein a decrease identifies the compound as being capable of inhibiting the activity. Therapeutic and diagnostic applications are also described.

FIGURE 1.

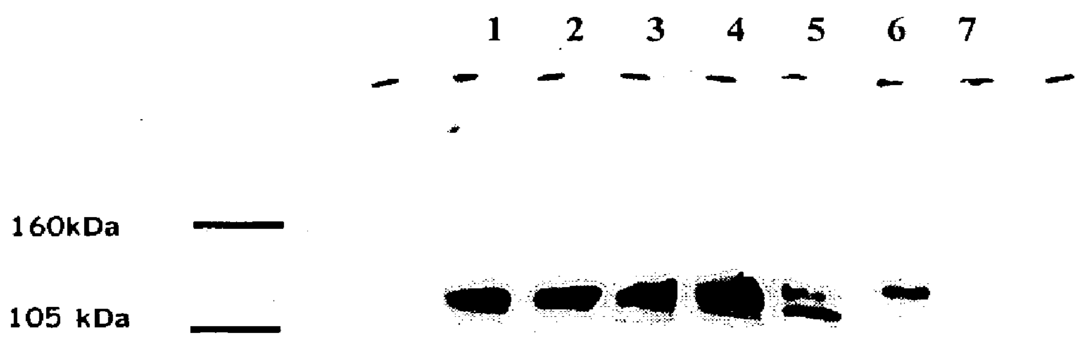


FIGURE 2.

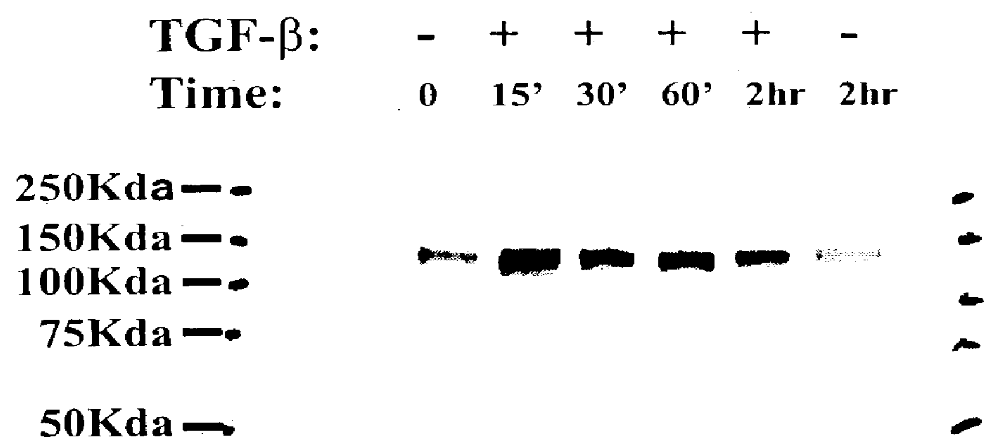


FIGURE 3.

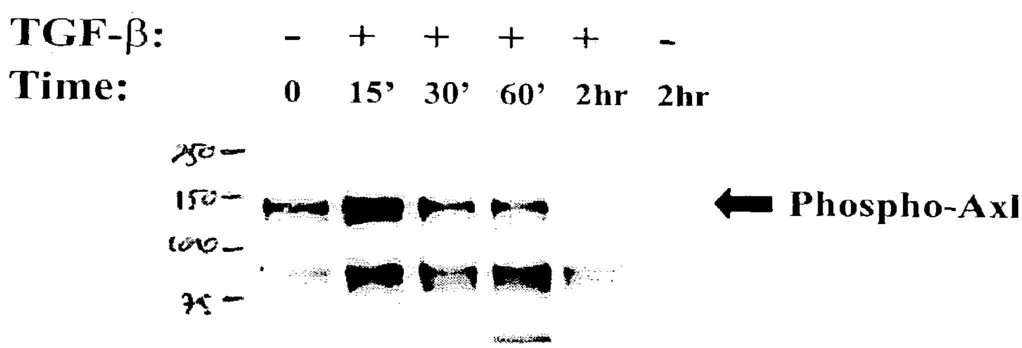
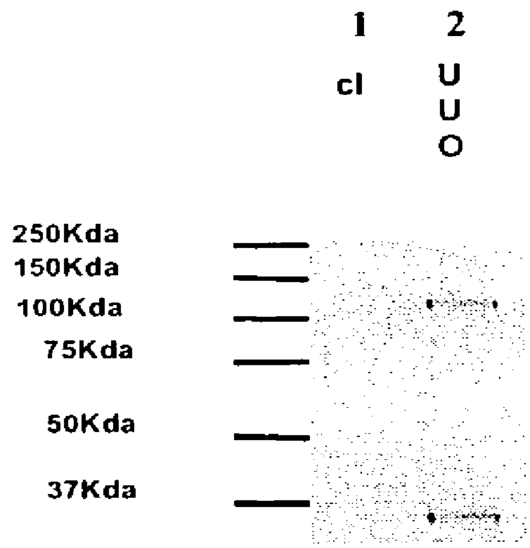


FIGURE 4.



USE OF AXL RECEPTOR FOR DIAGNOSIS AND TREATMENT OF RENAL DISEASE

[0001] This application claims the benefit of U.S. Provisional Application No. 60/356,374, filed Feb. 12, 2002.

FIELD OF THE INVENTION

[0002] The present invention relates to the identification and isolation of polynucleotide sequences, the expression of which is changed in various renal pathologies, and use of these isolated polynucleotides as probes for diagnosis, for screening of treatment modalities and as target for inactivation in fibrosis in general, and for kidney fibrosis and glomerulosclerosis, hallmarks of diabetic nephropathy, in particular.

BACKGROUND OF THE INVENTION

[0003] Accumulation of extracellular matrix and proliferation of fibroblasts are major hallmarks of fibrosis. Due to secretion of cytokines and growth factors, especially transforming growth factor beta (TGF-β), phenotypic change in fibroblast cells leads to increased deposition of extracellular matrix proteins. Repeated insults trigger up-regulation of tissue inhibitors of matrix metalloproteinases, favoring accumulation of extracellular matrix (Br J Surg 2001 11:1429-1441). Fibrosis is known to occur in many tissues (e.g., kidney, liver, lung, heart) in which injury or other specific stimulus causes acute inflammation at early stages, followed by scar formation and usually culminating in end-stage disease.

[0004] Cytokines are critical to a myriad of fundamental homeostatic and pathophysiological processes such as fever, wound healing, inflammation, tissue repair and fibrosis. They play important roles in regulating cell function such as proliferation, migration, and matrix synthesis. It is the balance or net effect of the complex interplay among these mediators and their downstream target proteins that appears to play a major role in regulating the initiation, progression and resolution of wounds and tissue fibrosis.

[0005] Diabetic Nephropathy

[0006] Diabetic nephropathy (hallmarks of which are glomerulosclerosis and renal fibrosis) is the single most prevalent cause of end-stage renal disease in the modern world, and diabetic patients constitute the largest population on dialysis. Such therapy is costly and far from optimal. Transplantation offers better outcome but suffers from a severe shortage of donors. More targeted therapies against diabetic nephropathy (as well as against other types of kidney pathologies) are not developed, since molecular mechanisms underlying these pathologies are largely unknown. Identification of a target essential functional gene that is modulated in the disease and affects the severity of the outcome of diabetes nephropathy has a diagnostic as well as therapuetic value.

[0007] It is known that many pathological processes in the kidney eventually culminate in similar or identical morphological changes, namely glomerulosclerosis and fibrosis. This means that different types of insults converge on the same single genetic program resulting in the proliferation of fibroblasts and overproduction by them of various protein components of connective tissue—two hallmarks of fibrosis. In addition, thickening of the basal membrane in the glomeruli accompanies interstitial fibrosis and culminates in glomerulosclerosis. Genes encoding proteins that are involved in kidney fibrosis and glomerulosclerosis may be roughly divided into two groups:

[0008] 1. genes, the expression of which lead to the triggering of these alterations; these may be specific to different pathological conditions.

[0009] 2. genes, the expression of which are responsible for the execution of the "fibrotic or sclerotic programs"; these may be common to all renal pathologies leading to fibrosis and glomerulosclerosis.

[0010] The identification of genes that belong to the second group should contribute to the understanding of molecular mechanisms that accompany fibroblast and mesangial cell proliferation and hypersecretion, and may constitute genetic targets for drug development aimed at preventing renal failure. Application of such drugs is expected to suppress, retard, prevent, inhibit or attenuate progression of fibrosis and glomerulosclerosis.

[0011] It is clear that the best way to assess the development of diabetic nephropathy is to characterize gene expression in established animal models of the disease. Examples of such models include (i) fa/fa rats, animals genetically deficient in leptin receptor that develop insulin resistant diabetes (type II diabetes) with progressive diabetic nephropathy, and (ii) GK rats, which are genetically manipulated, NIDDM phenotype rats. Another animal model in which the kidney fibrosis is evident but without a background of diabetes is unilateral ureteral obstruction (UUO) in which interstitial fibrosis is rapid and occurs within days following the obstruction.

[0012] Additional aspects of research may be based on an in vitro model system involving culture of human fibroblasts in vitro under conditions mimicking various parameters of the cell microenvironment existing in the diabetic kidney. These include treatment with high concentrations of glucose (modeling hyperglycemia), low concentrations of glucose, hypoxia (both modeling ischemic conditions that develop in the kidney following fibrosis and glomerulosclerosis) and TGF-β (one of the recognized pathogenic factors in fibrosis). Such a model system may complement the animal models in three important aspects:

[0013] 1. The system is fibroblast-specific; there is none of the interference often found in complex tissues that contain many cell types.

[0014] 2. The cells are of human origin (unlike the animal models).

[0015] 3. The insults are specific and of various concentrations and duration, thus enabling the investigation of both acute and chronic responses.

[0016] The Ax1 Receptor

[0017] Ax1 is a member of the receptor tyrosine kinase subfamily. It is an integral plasma membrane protein and has the unique structure of the extracellular region that juxtaposes Immunoglobulin-lambda (IgL) and FNIII domains and an intracellular region which contains an intracellular domain, part of which is the kinase domain. It can bind to the vitamin K-dependent protein Gas6, thereby transducing

signals into the cytoplasm. The extracellular domain of Ax1 can be cleaved and a soluble extracellular domain of 65 kDa can be released. Cleavage enhances receptor turnover, and generates a partially activated kinase (O'Bryan J P, Fridell Y W, Koski R, Varnum B, Liu E T. (1995) J Biol Chem. 270(2):551-557). However, the function of the cleaved domain is unknown.

[0018] Upon interaction with the Gas6 ligand, Ax1 becomes autophosphorylated, and a cascade of signal transduction events takes place. Known to be involved in this cascade are P13K, AKT, src, Bad, 14-3-3, PLC, ERK, S6K (mitogen-regulated kinase) and STAT (each of these was studied in different cell lines and/or systems).

[0019] Gas6, the ligand of Ax1, has a region rich with γ-carboxyglutamic acid (GLA domain) that allows for Ca^{++}-dependent binding to membrane phospholipids. Gas6is a weak mitogen and has an anti-apoptotic effect in NIH3T3 fibroblasts subjected to stress by TNF-induced cytotoxicity, or growth factor withdrawal. In NIH3T3 the binding of Gas6 to Ax1 results in activation of P13K, AKT, src and Bad.

[0020] In mesangial cells, Gas6 was found to have a mitogenic effect, thus demonstrating a possible function in the progression of glomerulosclerosis. Furthermore, it was recently shown (Yanagita M., Ishimoto Y., Arai H., Nagai K., Ito T., Nakano T., Salant D. J., Fukatsu A., Doi T. and Kita T. (2002) The Journal of Clinical Investigation 110 (2) 239-246), that Gas6 is an autocrine growth factor for mesangial cells, and that the anticoagulant wafarin together with the extracellular domain of Ax1 inhibit mesangial cell proliferation by specific blockade of the Gas6-mediated pathway in a mesangial-proliferative model of glomerulonephritis. Gas6 also promotes the survival of endothelial cells and is up-regulated from 6 h-72 h in the balloon-injured rat carotid artery (a model for arterial injury).

[0021] Angiotensin II, via its AT1 receptor, was shown to increase Ax1 mRNA and protein receptor in vascular smooth muscle cells (Melaragno M G, Wuthrich D A, Poppa V, Gill D, Lindner V, Berk B C, Corson M A. (1998) Circ Res. 83(7):697-704). The AT1 receptor antagonist losartan blocked the stimulatory effect of angiotensin on Ax1 expression. In the 32D myeloid cell line, expression of Ax1 permits aggregation of cells in response to Gas6 stimulation. This response does not require Ax1 kinase activity; thus, it was suggested that aggregation is mediated by a heterotypic intercellular mechanism whereby cell-bound Gas6 interacts with an Ax1 receptor on an adjacent cell.

[0022] Transgenic mice expressing the Ax1 receptor under the GM-CSF promoter exhibit phenotypic characteristics associated with non-insulin-dependent diabetes mellitus (NIDDM), including hyperglycemia and hyperinsulinemia, severe insulin resistance, progressive obesity, hepatic lipidosis, and pancreatic islet dysplasia. These mice were shown to express high levels of TNF-α. Ax1 proteolytic cleavage product (extracellular domain (ECD) of Ax1) created a more severe NIDDM phenotype in transgenic mice (Augustine K A, Rossi R M, Van G, Housman J, Stark K, Danilenko D, Varnum B, Medlock E. (1999) J Cell Physiol. 181(3):433-447). Ax1 has been shown to be involved in cellular adhesion, cell proliferation and regulation of homeostasis in the immune system (Lu Q and Lemke G (2001) Science 293(5528):306-311). Following Ax1 activation, the following phenomena have been observed: inhibition of apoptosis, increase in "normal" cell (non-transformed) survival of fibroblasts and endothelial cells, migration of Vascular Smooth Muscle Cell (VSMC) (inactivation of the Ax1 kinase blocks migration), enhancement of neointima formation in blood vessel wall (Melaragno M G, Fridell Y W, Berk B C. (1999) Trends Cardiovasc Med. (Review) 9(8):250-253) and involvement in lesion formation and the progression of atherosclerosis. Lack of Gas6 in knock out mice results in reduced nephrotoxicity following acute stimulation suggesting that Ax1, as the major ligand for GAS6 may be involved in this process in normal kidneys. Moreover, the mitogenic effect of GAS6 on mesangial cells may be carried out by signalling through Ax1.

[0023] What is Known About the Ax1 Gene:

Synonyms of Axl:	UFO, ARK (in mouse)

[0024] Structural information relating to the human Ax1 gene and gene product:

a.	Nucleotide Sequence:	5015 bp variant 1 gi:11863122 4986 bp variant 2 gi:11863124
b.	open reading frame:	894 aa (461–3145 bp)—variant 1 885 aa (459–3113 bp)—variant 2
c.	Protein sequence:	885 aa mw 140 kDa (human) gi:4502335

[0025] Domains: gi:4502335, performed by SMART:

a.	Extracellular region:	1–33 aa: signal peptide 41–136 and 145–224 aa: (Ig) 225–318 and 334–415 aa: 2 FNIII domains.
b.	Transmembrane domain:	441–463 aa
c.	Intracellular domain:	527–794 aa of SEQ ID NO:4

[0026] The intracellular domain contains a tyrosine kinase domain with motif Lys-Trp-Ile-Ala-Ile-Glu-Ser: SEQ ID NO:6 (present in all Tyro3 family members). Note that the intracellular domain has amino acid sequence SEQ ID NO:5.

[0027] Homology has been demonstrated to receptor tyrosine kinases of the Tyro3 family that includes besides Ax1 also Tyro3 (named also Sky or RSE), and MER proteins.

[0028] Tissue distribution: The inventors of the present invention, found that in mouse, Ax1 is expressed in distinct structures in a broad range of developing tissues in late embryogenesis and in cells forming organ capsules and connective tissue structures in adults.

[0029] Disease relevant patterns: Ax1 is a chronic myelogenous leukemia-associated oncogene and is also associated with colon cancer and melanoma.

[0030] Expression pattern during embryonic/fetal development: The Ax1 gene is evolutionarily conserved among vertebrate species, and is expressed during development in the mesenchyme.

[0031] The proliferation of mesangial cells seems to be an important pathological event that precedes glomerular sclerosis. Mesangial cells produce extracellular matrix and thus contribute to the fibro-sclerotic changes in the diabetic kidney. Gas6 was found to regulate mesangial cell proliferation through Ax1 in experimental glomerulonephritis. Inhibition of Gas6 interaction with Ax1 reduced proteinuria, mesangial cell proliferation, and restored renal function (Yanagita M et al., (1999) J Am Soc Nephrol 10:2503-2509; Yanagita M et al., (2001) Am J Pathol, 158:1423-1432). The following patent publications also relate to Ax1 or other tyrosine kinase receptors: U.S. Pat. No. 5,468,634; U.S. Pat. No. 6,087,144; U.S. Pat. No. 5,538,861; U.S. Pat. No. 5,968,508; U.S. Pat. No. 6,211,142; U.S. Pat. No. 6,235,769; WO 99/49894; WO 00/76309; WO 01/16181 and WO 01/32926.

[0032] Nowhere in the background art is it taught or suggested that modulation of the Ax1 receptor is useful for diagnosis and treatment of renal disease or, more specifically, diabetic nephropathy.

SUMMARY OF THE INVENTION

[0033] The main object of the present invention is the identification and isolation of novel genetic targets that may be used for development of drugs to treat fibrosis, as well as for development of diagnostic and prognostic applications. It is a further object of the present invention to identify and isolate novel genetic targets for development of drugs to treat renal disease, and more specifically to treat diabetic nephropathy, and using of such targets as a tool for diagnostic and prognostic applications. It is yet a further object of the present invention to identify and isolate novel genetic targets for development of drugs to treat the hallmarks of diabetic nephropathy, namely glomerulosclerosis and renal fibrosis.

[0034] The present invention provides these novel targets for development of novel therapeutic and diagnostic means via large-scale microarray-based analysis of gene expression in nephropathy and more specifically in diabetic nephropathy and kidney fibrosis models in vivo and in vitro. Preferably, the present invention identifies up- or down-regulator (responder) genes for gene therapy, diagnostics and therapeutics that have direct causal relationships between a fibrotic nephropathological disease and its related pathologies. More preferably, the present invention identifies the Ax1 gene as an up-regulator gene in the above-mentioned models.

[0035] The present invention further provides a process referred to herein as a screening assay for identifying modulators, i.e., candidate or compounds or agents including but not limited to neutralizing antibodies, peptides, peptido-mimetics, small molecules and other drugs, which bind to Ax1 or have an inhibitory effect on Ax1 expression or on Ax1 activity.

[0036] The compound or agent discovered by the above-mentioned screening assay that will inhibit signaling via the Ax1 receptor may be used in diabetic nephropathy to down-regulate mesangial cell proliferation and to slow the pace of or inhibit glomerulosclerosis or to reduce the proliferation of fibroblasts, to inhibit the accumulation of extracellular matrix and to reduce or limit the formation of fibrotic regions in the kidney. Preferably, the present invention identifies up- or down-regulator (responder) genes for gene therapy, diagnostics and therapeutics that have direct causal relationships between a disease and its related pathologies. More preferably, the present invention identifies the Ax1 gene for the above-mentioned uses.

BRIEF DESCRIPTION OF THE FIGURES

[0037] **FIG. 1**. This Figure demonstrates the endogenous Ax1 expression in response to TGF-β, in a variety of cell lines (Western blot analysis). Lane 1: NRK 49F cells; Lane2: NRK 49F cells with TGF-β 5 ng/ml, 24 hr; Lane 3: Rat1 cells; Lane4: Rat1 cells+TGF-β 5 ng/ml, 24 hr; Lane 5: W138 cells; Lane 6: HeLa cells; Lane 7: 293 cells.

[0038] **FIG. 2**. This Figure demonstrates that Ax1 protein is up-regulated in rat1 cells in response to TGF-β. Total cell lysates from Rat1 cells exposed to TGF-β stimulation (5 ng/ml for 15 minutes, 30 minutes, 60 minutes and 2 hours) were run on gel and probed with anti Ax1 C20 Ab (Western blot analysis). Samples in the first and last lane are from cells without TGF-β stimulation.

[0039] **FIG. 3**. This Figure demonstrates that TGF-β-dependent induction of Ax1 is accompanied by increase in phosphorylated-Ax1 levels. Rat1 cells exposed to TGF-β stimulation (5 ng/ml for 15 minutes, 30 minutes, 60 minutes and 2 hours) were used for immunoprecipitation. Ax1 was immunoprecipited with anti Ax1 M-20. Anti phosphotyrosine antibodies were used to monitor ax1 phosphorylation state following TGF-β treatment (5 ng/ml for 15 minutes-2 hr). Samples in the first and last lane are from cells without TGF-β stimulation.

[0040] **FIG. 4**. This Figure demonstrates up regulation of the Ax1 polypeptide following UUO in Rat. Expression of Ax1 protein was monitored in normal SD rat kidneys following UUO model. Lane 1: Ax1 expression in contralateral (cl) non-treated kidneys; Lane 2: Ax1 expression in obstructed kidneys (UUO, for 25 days).

DETAILED DESCRIPTION OF THE INVENTION

[0041] According to the present invention, purified, isolated and cloned nucleic acid sequences, specifically the nucleic acid sequence that encodes the Ax1 receptor, associated with nephropathy and more specifically with diabetic nephropathy and with fibrotic and glomerulosclerotic kidneys and having sequences as specified herein or having complementary or allelic sequence variations thereto, are disclosed. Furthermore, a purified, isolated and cloned nucleic acid sequence associated with nephropathy and having a sequence which encodes SEQ ID NO: 2 and 4 herein is also disclosed. The database provides two transcript variants:

[0042] transcript variant 1: NM_021913 GI:11863122

[0043] transcript variant 2: NM_001699 GI:11863124

[0044] As used herein, the term "Ax1 gene " is defined as any homolog of the Ax1 gene having preferably 90% homology, more preferably 95% homology, and even more preferably 98% homology to the amino acid encoding region of SEQ ID NO:1 and NO:3 or nucleic acid sequences which

bind to the Ax1 gene under conditions of highly stringent hybridization, which are well-known in the art (for example Ausubel et al., Current Protocols in Molecular Biology, John Wiley and Sons, Baltimore, Md. (1988), updated in 1995 and 1998). Note that 18 nucleotides upstream of the ATG in both SEQ ID NO:1 and NO:3 are not in the amino acid encoding region, and many nucleotides downstream of the stop signal are also not in the amino acid encoding region.

[0045] As used herein, the term "Ax1" or "Ax1 polypeptide" or "Ax1 receptor" is defined as any homolog of the Ax1 polypeptide having preferably 90% homology, more preferably 95% homology, and even more preferably 98% homology to SEQ ID NO:2, to SEQ ID NO:4, or to SEQ ID NO:5 as either full-length or a fragments or a domain thereof, as a mutant or the polypeptide encoded by a spliced variant nucleic acid sequence, as a chimera with other polypeptides, provided that any of the above has the same or substantially the same biological function as the Ax1 receptor. Ax1 polypeptide, or an Ax1 polypeptide homolog, may be present in different forms, including but not limited to soluble protein, membrane-bound (either in purified membrane preparations or on a cell surface), bead-bound, or any other form presenting Ax1 protein or fragments and polypeptides derived thereof. The Ax1 polypeptide or Ax1 receptor comprises the intracellular domain represented by SEQ ID NO:5. Particular fragments of the Ax1 polypeptide include amino acids 1-50, 51-100, 101-150, 151-200, 201-250, 251-300, 301-350, 351-400, 401-450, 451-500, 501-550, 551-600, 601-650, 651-700, 701-750, 751-800 and 801-850, of SEQ ID NOS: 2 and 4, and amino acids 851-894 and 851-885 of SEQ ID NOS: 2 and 4 respectively. Further particular fragments of the Ax1 polypeptide include amino acids 25-74, 75-124, 125-174, 175-224, 225-274, 275-324, 325-374, 375-424, 425-474, 475-524, 525-574, 575-624, 625-674, 675-724, 725-774, 775-824 and 825-874 of SEQ ID NOS: 2 and 4 and amino acids 875-894 and 875-885 of SEQ ID NOS: 2 and 4 respectively.

[0046] It is also envisaged by the instant invention that inhibition of any other members of the Tyro3 family, which includes Tyro3, Ax1 and Mer, may have therapeutic results similar to those observed by inhibition of Ax1.

[0047] Where the sequences are partial sequences, they may be used as markers/probes for genes 1 5 that are up-regulated in fibrosis. In general these partial sequences which are designated "Expressed Sequence Tags" (ESTs), are markers for the genes actually expressed in vivo, and are ascertained as described herein in the Examples section. Generally, ESTs comprise DNA sequences corresponding to a portion of nuclear encoded mRNA. The EST has a length that allows for polymerase chain reaction (PCR), and is used as a hybridization probe, with a unique designation for the gene with which it hybridizes (generally under conditions sufficiently stringent to require at least 95% base pairing). For a detailed description and review of ESTs and their functional utility see WO 93/00353 which is incorporated herein in its entirety by reference WO 93/00353 further describes how the EST sequences can be used to identify the transcribed genes.

[0048] As used herein, a "target molecule" is a molecule with which Ax1 or an Ax1 gene family member binds or interacts or phosphorylates or activates in nature; for example, a molecule on the surface of a cell that expresses Ax1, a molecule on the surface of a second cell, a molecule associated with the internal surface of a cell membrane or a cytoplasmic molecule. An Ax1 target molecule is mainly a component of a signal transduction pathway that facilitates transduction of an extracellular signal from Ax1 (e.g., a signal generated by the binding of a ligand of Ax1to the membrane-bound Ax1 molecule) through the cell membrane and into the cell. The target, for example, can be a second intercellular protein that mediates downstream signaling from Ax1.

[0049] As used herein, the term "compound" is defined as comprising any small chemical molecule, antibodies, neutralizing antibodies, antisense DNA or RNA molecules, mRNA, proteins, polypeptides and peptides including peptido-mimetics and dominant negatives, and expression vectors.

[0050] In one embodiment, the invention provides assays for screening candidates or compounds that bind to, modulate the activity of, or modulate the expression level of Ax1. The compounds of the present invention can be obtained using any of the numerous approaches in combinatorial and non-combinatorial library methods known in the art, including biological libraries (proteins, peptides, etc:), spatially addressable parallel solid phase or solution phase libraries, synthetic library methods, and natural product libraries.

[0051] The modulator of Ax1 expression (transcription or translation) or polypeptide activity may be inter alia a small chemical molecule which generally has a molecular weight of less than 2000 daltons, more preferably less than 1000 daltons, even more preferably less than 500 daltons. Other modulators may be antibodies preferably neutralizing antibodies or fragments thereof including single chain antibodies, antisense oligonucleotides, antisense DNA or RNA molecules, proteins, polypeptides and peptides including peptido-mimetics and dominant negatives, and expression vectors. These modulators may act as follows: small molecules may affect expression and/or activity; antibodies—only activity; all kinds of antisense—will effect Ax1 expression; dominant negative and peptidomimetics—only activity; expression vectors may be used inter alia for delivery of antisense or dominant-negative.

[0052] Approaches have recently been developed that utilize small molecules, which can bind directly to proteins and can be used to alter protein function (for review see B. R. Stockwell, (2000) Nature Reviews/Genetics, 1, 116-125). As mentioned above, low molecular weight organic compounds can permeate the plasma membrane of target cells relatively easily and, therefore, methods have been developed for their synthesis. These syntheses, in turn, have yielded libraries that contain ligands for many proteins. Recent developments have brought a greatly increased variety of creatively selected, novel, small organic molecules that will function as powerful tools for perturbing biological systems. Such small molecules can be used to activate or inactivate specific members of a protein family.

[0053] Examples of methods for the synthesis of molecular libraries can be found in the art, for example, in DeWitt et al. (1993) Proc. Natl. Acad. Sci. U.S.A. 90:6909; Erb et al. (1994) Proc. Natl. Acad. Sci. USA 91:11422; Zuckermann et al. (1994). J. Med. Chem. 37:2678; Cho et al. (1993) Science 261:1303; Carrell et al. (1994) Angew. Chem. Int.

Ed. Engl. 33:2059; Carell et al. (1994) Angew. Chem. Int. Ed. Engl. 33:2061; and in Gallop et al. (1994) J. Med. Chem. 37:1233.

[0054] Libraries of compounds may be presented in solution (Houghten (1992) Biotechniques 13:412-421), or on beads (Lam (1991) Nature 354:82-84), chips (Fodor (1993) Nature 364:555-556), bacteria (Ladner U.S. Pat. No. 5,223,409), spores (Ladner U.S. Pat. No. 5,223,409) plasmids (Cull et al. (1992) Proc. Natl. Acad. Sci. (USA) 89:1865-1869) or on bacteriophage (Scott and Smith (1990) Science 249:386-390; Devlin (1990) Science 249:404-406; Cwirla et al. (1990) Proc. Natl. Acad. Sci. 87:6378-6382; Felici (1991) J. Mol. Biol. 222:301-310).

[0055] In accordance with another embodiment of the present invention, an assay is a cell-based assay in which cells of mammalian origin are transfected with a kinase active Ax1 construct. The cells are contacted with a compound; the ability of the compound to inhibit Ax1 activity is determined.

[0056] Yet, in another embodiment, the assay is comprised of incubating cells over-expressing active Ax1 with a second molecule preferably an Ax1 target, to form an assay mixture. This assay mixture is then incubated with a compound identified according to any of the screening processes of the present invention, and the ability of the identified compound to inhibit Ax1 activity towards its target is determined.

[0057] Thus in this embodiment the ability of the identified compound to interact with Ax1 is determined by measuring the ability of the identified compound to preferentially bind to Ax1 as compared to the Ax1 target molecule i.e. the second compound (i.e. measurement of competitive binding).

[0058] In another embodiment, an assay is a cell-based assay comprising contacting cells expressing an Ax1 receptor or fragment thereof, with a compound and determining the ability of the compound to modulate (i.e., stimulate or inhibit) the activity of Ax1. Determining the ability of the compound to modulate the activity of Ax1 can be accomplished, for example, by determining the enzymatic activity of Ax1. The latter can be accomplished directly by following tyrosine phosphorylation of cellular proteins downstream to Ax1 (or Ax1 target molecules) or by a reporter based assay based on measuring, for example, metabolically labeling Ax1-expressing cells with radioactive (either ^{32}P or ^{33}P) phosphate and following the accumulation of radioactivity in phosphotyrosine-specific immunoprecipitates of cells stimulated by the Ax1 target molecule or by using fluorescence polarization for the detection of Ax1 activity.

[0059] Alternatively, determining the activity of Ax1 can be accomplished indirectly by detecting induction of a cellular second messenger of Ax1 and/or its downstream effectors (i.e., increases in intracellular free Ca^{2+} ion, diacylglycerol production, IP_3 generation, etc.), detecting catalytic/enzymatic activity of the target using an appropriate endogenous or exogenous substrate, detecting the induction of a reporter gene (comprising an Ax1-responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, e.g., luciferase), or detecting a cellular response, for example, cell survival, cellular differentiation, or cell proliferation.

[0060] In yet another embodiment, an assay is a cell-free assay comprising incubating recombinant Ax1, or fragments thereof, with a compound and determining the ability of the compound to bind to Ax1. Binding of the compound to Ax1 can be determined either directly or indirectly as described above. For example, the assay comprises incubating Ax1 with a known compound that binds Ax1, or an Ax1 target molecule, to form an assay mixture. This assay mixture is further incubated with a compound and the ability of the compound to preferentially bind to Ax1 as compared to the known compound (or target molecule) is measured.

[0061] Yet, in another embodiment of the present invention, an assay is a cell-free assay comprising incubating Ax1 with a compound and determining the ability of the compound to modulate (e.g., stimulate or inhibit) the activity of Ax1. Determining the ability of the compound to modulate the activity of Ax1 can be accomplished by following auto phosphorylation of Ax1 or by following tyrosine phosphorylation of Ax1 substrates by, for example, performing in vitro kinase assays using radioactively-labeled (either ^{32}P or ^{33}P) ATP and measuring the accumulation of radioactivity in the phosphorylated substrate, or by using fluorescence polarization using, for example, the commercially available Molecular Devices kit.

[0062] The cell-free assays of the present invention are compatible with the use of either a soluble form, a membrane-bound form or an immobilized form of Ax1. In the case of cell-free assays comprising the membrane-bound form of Ax1, it may be desirable to utilize a solubilizing agent such that the membrane-bound form of Ax1 is maintained in solution. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton™ X-100, Triton™ X-114, 3-[(3-cholamidopropyl)dimethylamino]-1-propane sulfonate (CHAPS), or 3-[(3-cholamidopropyl)dimethylamino]-2-hydroxy-1-propane sulfonate (CHAPSO).

[0063] In some of the embodiments of the above assay processes, it may be desirable to immobilize either Ax1 or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a compound to Ax1, or interaction of Ax1 with a target molecule in the presence and/or absence of a compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtitre plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows one or both of the proteins to bind to a matrix. For example, glutathione-S-transferase/Ax1 fusion proteins or glutathione-S-transferase/target fusion proteins can be adsorbed onto glutathione Sepharose beads or glutathione derivatized microtitre plates, which are then combined with the compound and either the non-adsorbed target protein or Ax1, and the mixture incubated under conditions suitable for complex formation. Following incubation, the beads or microtitre plate wells are washed to remove any unbound components, the matrix is immobilized in the case of beads, and complex formation is determined either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix and the level of Ax1 binding or activity determined using standard techniques.

[0064] Other techniques for immobilizing proteins on matrices can also be used in the screening assays of the invention. For example, either Ax1 or its target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated Ax1 or target molecules can be prepared from biotin-NHS (N-hydroxysuccinimide) using techniques well known in the art (e.g., biotinylation kit, Pierce Chemicals, Rockford, Ill.), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical, Rockford, Ill.). Alternatively, antibodies reactive with Ax1 or target molecules but which do not interfere with binding of Ax1 to its target molecule can be bound to the wells of the plate, and free target or Ax1 trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with Ax1 or target molecule, as well as enzyme-linked assays which rely on detecting an enzymatic activity of Ax1 or that associated with Ax1 or its target molecule.

[0065] In another embodiment, modulators of Ax1 expression are identified in a process wherein cells are contacted with a compound and the expression of Ax1 mRNA or protein in the cell sample is determined. The level of expression of Ax1 mRNA or protein in the presence of the candidate compound is compared to the level of expression of Ax1 mRNA or protein in the absence of the candidate compound. The candidate compound can then be identified as a modulator of Ax1 expression based on this comparison. For example, when expression of Ax1 mRNA or protein is greater in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of Ax1 mRNA or protein expression. Alternatively, when expression of Ax1 mRNA or protein is lower in the presence of the compound than in its absence, the candidate compound is identified as an inhibitor of Ax1 mRNA or protein expression. The level of Ax1 mRNA or protein expression in the cells can be determined by methods described herein for detecting Ax1 mRNA or protein. A preferred embodiment of the present invention provides for a process of identifying a compound capable of inhibiting the activity of a human Ax1 receptor that comprises the steps of:

[0066] (i) contacting the Ax1 receptor or cells expressing the Ax1 receptor with the compound;

[0067] (ii) measuring the Ax1 receptor activity in the presence of the compound; and

[0068] (iii) comparing the activity measured in step (ii) to that measured in the absence of the compound under controlled conditions, wherein a decrease identifies the compound as being capable of inhibiting the activity.

[0069] In one embodiment of the invention, the activity measured in the above mentioned process is tyrosine phosphorylation of a substrate of the Ax1 receptor or auto phosphorylation of the Ax1 receptor. In another embodiment the cells that are contacted with the compound are mesangial cells and the activity measured is proliferation of said mesangial cells or the cells contacted with the compound are renal fibroblasts and the activity measured is proliferation of said renal fibroblasts. In further embodiment, the cells contacted with the compound are renal fibroblasts and the activity measured is collagen deposition in the extracellular matrix of said renal fibroblasts. In a further embodiment, the cells contacted with the compound are renal tubular cells and the activity measured is proliferation of said renal tubular cells. Yet, in another embodiment, the cells contacted with the compound are renal tubular cells and the activity measured is transdifferentiation to myofibroblasts.

[0070] In another embodiment of present invention, the cells in the contacting step (i) of the above mentioned process have previously been transfected by the Ax1 gene, either transiently or stably transfected. Yet, in another embodiment, the controlled conditions in step (iii) comprises measurement upon contacting cells which lack an active Ax1 gene. In a further embodiment, the controlled conditions in step (ii) comprise comparison upon contacting similar cells having the absence of an active Ax1 gene or similar cells having a mutated inactive form of the Ax1 gene. In another embodiment of the present invention, the Ax1 receptor of the above mentioned processes comprises consecutive amino acids, the sequence of which is set forth either in SEQ ID NO:5, or SEQ ID NO:2 or SEQ ID NO:4. In a further embodiment, the Ax1 receptor comprises a biologically active portion of the intracellular domain.

[0071] In another embodiment of the present invention, the compound identified according to any of the processes mentioned in the above, inhibits the activity of the Ax1 receptor at least 2-fold, more preferably 5-fold, even more preferably 100-fold and most preferably 200-fold, more effectively than it inhibits the activity of the tyrosine kinase receptors FGFR1, VER4, KIN24, HGFr, met, EGFR, IGF-1r, InsR and Ab1.

[0072] Yet, in a further embodiment of the invention, a compound identified according to the processes of the above can be used in the preparation of a medicament for therapy of nephropathy.

[0073] In a further embodiment of the invention, prior to contacting the Ax1 receptor or cells expressing the Ax1 receptor with the compound, the Ax1 receptor is contacted with a second compound known to bind Ax1. In another embodiment, either the Ax1 receptor or the second compound are immobilized.

[0074] An embodiment of the present invention provides for a process of identifying a compound capable of decreasing the level of an Ax1 gene expression that comprises the steps of:

[0075] (i) contacting cells capable of expressing an Ax1 receptor with the compound;

[0076] (ii) measuring the expression level of the Ax1 gene in the presence of the compound; and

[0077] (iii) comparing the level measured in step (ii) to that measured in the absence of the compound, under controlled conditions, wherein a decrease identifies the compound as being capable of inhibiting the activity.

[0078] In another embodiment, the cells in the contacting step (i) of the above mentioned process have been transfected by the Ax1 gene, either transiently or stably transfected. Yet, in another embodiment, the controlled conditions in step (iii) comprises comparison upon contacting identical cells in the absence of the chemical compound. In a further embodiment, the controlled conditions in step (ii) comprises comparison upon contacting similar cells having

the absence of an active Ax1 gene or the similar cells having a mutated inactive form of the Ax1 gene. In another embodiment, prior to step (ii), the cells of step (i) are exposed to at least one insult that is related to nephropathy. The insult may be selected from the group consisting of hyperglycemia, hypoxia, low glucose concentration, and TGF–. In accordance with the invention, the cells exposed to the compound can be selected from the group consisting of mesangial cells, renal fibroblasts, and renal tubular cells. Yet, in a further embodiment of the invention, a compound identified according to the above mentioned process can be used in the preparation of a medicament for therapy of nephropathy.

[0079] It is the subject of the present invention further to provide for a method of diagnosing nephropathy in a subject comprising determining, in a sample from the subject, the level of an Ax1 receptor encoding polynucleotide, wherein a higher level of the polynucleotide compared to the level of the polynucleotide in a subject free of nephropathy is indicative of nephropathy. In one embodiment, the diagnosed nephropathy is diabetic nephropathy or kidney fibrosis, and the sample is taken from kidney tissue.

[0080] This application is also directed to a process of identifying a compound capable of inhibiting the activity of a human Ax1 receptor by screening a plurality of compounds that comprises the steps of:

[0081] (i) contacting the Ax1 receptor or cells expressing the Ax1 receptor with the plurality of compounds;

[0082] (ii) measuring the Ax1 receptor activity in the presence of the plurality of compounds;

[0083] (iii) comparing the activity measured in step (ii) to that measured in the absence of the plurality of compounds under controlled conditions, wherein a decrease identifies the plurality of compounds as being capable of inhibiting the activity; and

[0084] (iv) separately determining which compound or compounds present in the plurality inhibit the activity of a human Ax1 receptor.

[0085] In yet another aspect of the invention, Ax1 protein can be used as "bait protein" in a two-hybrid assay or three-hybrid assay (e.g., U.S. Pat. No. 5,283,317; Zervos et al. (1993) Cell 72:223-232; Madura et al. (1993) J. Biol. Chem. 268:12046-12054; Bartel et al. (1993) Biotechniques 14:920-924; Iwabuchi et al. (1993) Oncogene 8:1693-1696; and Brent WO 94/10300), to identify other proteins which bind to or interact with Ax1 ("Ax1-binding proteins") and modulate Ax1 activity. Such Ax1-binding proteins are also likely to be involved in the propagation of signals by Ax1 as, for example, upstream or downstream elements of the Ax1 signaling pathway.

[0086] The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In the first construct, the gene that codes for Ax1 is fused to a gene encoding the DNA binding domain of a known transcription factor (e.g., GAL-4). In the second construct, a DNA sequence obtained from a library of DNA sequences that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact in vivo, forming an Ax1-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (e.g., LacZ) which is linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene that encodes the protein that interacts with Ax1.

[0087] This invention further pertains to novel agents identified by the above-described screening assays and uses thereof for treatments of renal disease and more specifically for the treatment of nephropathy, especially diabetic nephropathy as described herein.

[0088] The present invention further provides a process for identifying a compound capable of decreasing the level of Ax1 gene expression useful for therapy of nephropathy. According to that process cells capable of expressing the Ax1 receptor are contacted with a compound, followed by exposing the cells to at least one insult or pathological parameter that is related to nephropathy. Comparison of the level of Ax1 gene expression to that obtained by a control can indicate the inhibitory effect of said compound on the Ax1 activity.

[0089] The present invention further provides transgenic animals and cell lines carrying at least one expressible gene, particularly that encoding the Ax1 receptor, identified by the present invention. The present invention further provides knock-out eucaryotic organisms, in which at least one nucleic acid sequence, as identified by the probes of the present invention and prepared as described below, was knocked out.

[0090] The present invention provides a process for discovering drugs for use in treating nephropathy in a patient in need of such treatment. These drugs, in therapeutically effective amounts, will be antagonists of at least one protein, particularly the Ax1 receptor, as encoded by the nucleic acid sequences or as presented by the amino acid sequences identified herein or by the probes of the present invention. Although these drugs are preferentially directed to treatment of kidney fibrosis, they may also be useful for the treatment of other fibrotic diseases, such as liver, lung and heart. These drugs may also be used to treat or prevent restenosis, i.e., to prevent or reduce proliferation of smooth muscle cells. These drugs may also be used as anti-angiogenic drugs for the treatment of cancer and other conditions where preventing or reducing proliferation of endothelial cells is desired.

[0091] Any of the screening assays according to the present invention can include a step of identifying the compound (as described above) which tests positive in the assay, and can also include the further step of producing as a medicament that which has been so identified. It can also include steps of improving the compound to increase its desired activity before incorporating the improved compound into a medicament. It is considered that medicaments comprising such compounds are part of the present invention.

[0092] The present invention further provides for a process of preparing a composition which comprises:

[0093] (i) identifying a compound that inhibits activity of a human Ax1 receptor by at least one of the above processes ; and

[0094] (ii) admixing said compound with a carrier.

[0095] In one embodiment of the invention, the carrier of the above mentioned process is a pharmaceutically effective carrier, and the compound admixed with the carrier is present in a pharmaceutically effective amount.

[0096] Additionally, the present invention provides a method of regulating fibrosis-associated pathologies in a patient in need of such . treatment by administering to a patient a therapeutically effective amount of at least one antisense (AS) oligonucleotide against the nucleic acid sequences or dominant negative peptide directed against the Ax1 sequences or Ax1 proteins.

[0097] As used herein, "negative dominant peptide" refers to a partial cDNA sequence that encodes a part of a protein, i.e., a peptide (Herskowitz I. (1987) Nature (Review) 329(6136):219-222). This peptide can have a function different from that of the protein from which it was derived. It can interact with a wild type protein target and inhibit its activity or it can interact with other proteins and inhibit their activity in response to the wild type target protein. Specifically, negative dominant refers to the ability of a peptide to inhibit the activity of a natural protein normally found in the cell in order to modulate the cellular phenotype, i.e., making the cell more resistant or sensitive to killing. For therapeutic intervention either the peptide itself is delivered as the active ingredient of a pharmaceutical composition or the cDNA can be delivered to the cell utilizing the same methods as for AS delivery.

[0098] The antagonist/regulating agent/active ingredient is dosed and delivered in a pharmaceutically acceptable carrier as described herein below. As used herein, the term "antagonist or antagonizing" is understood in its broadest sense. Antagonism can include any mechanism or treatment that results in inhibition, inactivation, blocking or reduction in gene activity or gene product. It should be noted that the inhibition of a gene or gene product may provide for an increase in a corresponding function that the gene or gene product was regulating. The antagonizing step can include blocking cellular receptors for the gene products and can include AS treatment as discussed below.

[0099] Many reviews have covered the main aspects of AS technology and its enormous therapeutic potential (Anazodo et al. (1995) Gene 166(2):227-232). There are reviews on the chemical (Crooke S T (1995)Hematol Pathol. (Review) 9(2):59-72; Uhlmann et al.(2000) Methods Enzymol. 313:268-284.), cellular (Wagner R W (1994) Nature (Review) 372(6504):333-335), and therapeutic (Hanania et al. (1995) Am J Med.(Review) 99(5):537-552; Scanlon et al. (1995) FASEB J. (Review) 9(13):1288-1296; Gewirtz AM (1993) Leuk Lymphoma. 1993;11 Suppl 1:131-137) aspects of this rapidly developing technology. Within a relatively short time, ample information has accumulated about the in vitro use of AS nucleotide sequences in cultured primary cells and cell lines, as well as the in vivo administration of such nucleotide sequences for suppressing specific processes and changing body functions in a transient manner. AS intervention in the expression of specific genes can be achieved by the use of synthetic AS oligonucleotide sequences (for recent reports see Lefebvre-d'Hellencourt et al. (1995) Eur Cytokine Netw. (Review) 6(1):7-19; Agrawal S (1996) Trends Biotechnol.(Review) 14(10):376-387; Lev-Lehman et al. (1997) Blood 89(10):3644-3653. Instead of an AS sequence as discussed herein above, ribozymes may be utilized. This is particularly necessary in cases where AS therapy is limited by stoichiometric considerations (Sarver et al. (1990) Gene Regulation and Aids, pp. 305-325). Ribozymes can then be used that will target the same sequence. Ribozymes are RNA molecules that possess RNA catalytic ability (see Cech T R (1993) Gene (Review) 135(1-2):33-36) and that cleave a specific site in a target RNA molecule.

[0100] The ribozyme type utilized in the present invention is selected as is known in the art. Hairpin ribozymes are now in clinical trial and are the preferred type. In general the ribozyme is from 30-100 nucleotides in length.

[0101] Modifications or analogs of nucleotides can be introduced to improve the therapeutic properties of the nucleotides. Improved properties include increased nuclease resistance and/or increased ability to permeate cell membranes.

[0102] Nuclease resistance, where needed, is provided by any method known in the art that does not interfere with biological activity of the AS oligodeoxy-nucleotides, cDNA and/or ribozymes as needed for the method of use and delivery (Eckstein F (1985) Annu Rev Biochem. (Review) 54:367-402; Spitzer S and Eckstein F (1988) Nucleic Acids Res. 16(24):11691-11704; Woolf et al. (1990) Nucleic Acids Res. 18(7):1763-1769) . Modifications that can be made to oligonucleotides in order to enhance nuclease resistance include modifying the phosphorous or oxygen heteroatom in the phosphate backbone. These include preparing methyl phosphonates, phosphorothioates, phosphorodithioates and morpholino oligomers. One embodiment provides for phosphorothioate bonds linking between the four to six 3'-terminus nucleotide bases. Alternatively, phosphorothioate bonds link all the nucleotide bases. Other modifications known in the literature may be used where the biological activity is retained, but the stability to nucleases is substantially increased.

[0103] The present invention also includes all analogs of, or modifications to, an polynucleotide or oligonucleotide of the invention that does not substantially affect the function of the polynucleotide or oligonucleotide. The nucleotides can be selected from naturally occurring or synthetically modified bases. Naturally occurring bases include adenine, guanine, cytosine, thymine and uracil. Modified bases of the oligonucleotides include xanthine, hypoxanthine, 2-aminoadenine, 6-methyl-, 2-propyl- and other alkyl-adenines, 5-halo uracil, 5-halo cytosine, 6-aza cytosine and 6-aza thymine, pseudo uracil, 4-thiuracil, 8-halo adenine, 8-aminoadenine, 8-thiol adenine, 8-thiolalkyl adenines, 8-hydroxyl adenine and other 8-substituted adenines, 8-halo guanines, 8-amino guanine, 8-thiol guanine, 8-thioalkyl guanines, 8-hydroxyl guanine and other substituted guanines, other aza and deaza adenines, other aza and deaza guanines, 5-trifluoromethyl uracil and 5-trifluoro cytosine.

[0104] In addition, analogs of nucleotides can be prepared wherein the structures of the nucleotides are fundamentally

altered and are better suited as therapeutic or experimental reagents. An example of a nucleotide analog is a peptide nucleic acid (PNA) wherein the deoxyribose (or ribose) phosphate backbone in DNA (or RNA) is replaced with a polyamide backbone similar to that found in peptides. PNA analogs have been shown to be resistant to degradation by enzymes and to have extended lives in vivo and in vitro. Further, PNAs have been shown to bind more strongly to a complementary DNA sequence than to a DNA molecule. This observation is attributed to the lack of charge repulsion between the PNA strand and the DNA strand. Other modifications that can be made to oligonucleotides include polymer backbones, cyclic backbones, or acyclic backbones.

[0105] The active ingredients of the pharmaceutical composition can include oligonucleotides that are nuclease resistant, needed for the practice of the invention, or a fragment thereof shown to have the same effect targeted against the appropriate sequence(s) and/or ribozymes. Combinations of active ingredients as disclosed in the present invention can be used, including combinations of AS sequences.

[0106] The AS oligonucleotides (and/or ribozymes) and cDNA of the present invention can be synthesized by any method known in the art for ribonucleic or deoxyribonucleic nucleotides. For example, an Applied Biosystems 380B DNA synthesizer can be used. When fragments are used, two or more such sequences can be synthesized and linked together for use in the present invention.

[0107] The nucleotide sequences of the present invention can be delivered either directly or with viral or non-viral vectors. When delivered directly the sequences are generally rendered. nuclease resistant. Alternatively the sequences can be incorporated into expression cassettes or constructs such that the sequence is expressed in the cell as discussed herein below. Generally the construct contains the proper regulatory sequence or promoter to allow the sequence to be expressed in the targeted cell.

[0108] The polypeptides of the present invention may be produced recombinantly (see generally Marshak et al., 1996 "Strategies for Protein Purification and Characterization. A laboratory course manual." Plainview, N.Y.: Cold Spring Harbor Laboratory Press, 1996) and analogs may be produced by post-translational processing. Differences in glycosylation can provide polypeptide analogs.

[0109] As used herein, the term "polypeptide" refers to, in addition to a polypeptide, a peptide and a full protein, as well as a fragment or fragments thereof

[0110] As used herein, "functionally relevant" refers to the biological property of the molecule and in this context means an in vivo effector or antigenic function or activity that is directly or indirectly performed by a naturally occurring polypeptide or nucleic acid molecule. Effector functions include but are not limited to receptor binding, any enzymatic activity or enzyme modulatory activity, any carrier binding activity, any hormonal activity, any activity in promoting or inhibiting adhesion of cells to extracellular matrix or cell surface molecules, or any structural role, as well as having the nucleic acid sequence encode functional protein and be expressible. The antigenic functions essentially mean the possession of an epitope or an antigenic site that is capable of cross-reacting with antibodies raised against a naturally occurring protein. Biologically active analogs share an effector function of the native polypeptide that may, but need not, in addition possess an antigenic function.

[0111] In diagnosis, the sample is taken from a bodily fluid or from a tissue, preferably kidney tissue; the bodily fluid is selected from the group of fluid consisting of blood, lymph fluid, ascites, serous fluid, pleural effusion, sputum, cerebrospinal fluid, lacrimal fluid, synovial fluid, saliva, stool, sperm and urine, preferably blood or urine. Measurement of level of the Ax1 polypeptide may be determined by a method selected from the group consisting of immunohistochemistry, western blotting, ELISA, antibody microarray hybridization and targeted molecular imaging. Such methods are well-known in the art, for example for immunohistochemistry: M. A. Hayat (2002) Microscopy, Immunohistochemistry and Antigen Retrieval Methods: For Light and Electron Microscopy, Kluwer Academic Publishers; Brown C (1998): "Antigen retrieval methods for immunohistochemistry", *Toxicol Pathol;* 26(6): 830-1); for western blotting: Laemmeli U K(1970): "Cleavage of structural proteins during the assembly of the head of a bacteriophage T4", *Nature;*227: 680-685; and Egger & Bienz(1994) "Protein (western) blotting", *Mol Biotechnol;* 1(3): 289-305); for ELISA: Onorato et al.(1998) "Immunohistochemical and ELISA assays for biomarkers of oxidative stress in aging and disease",*Ann NY Acad Sci* 20; 854: 277-90); for antibody microarray hybridization :Huang(2001) "Detection of multiple proteins in an antibody-based protein microarray system, *Immunol Methods* 1; 255 (1-2): 1-13) ; and for targeted molecular imaging:Thomas (2001). Targeted Molecular Imaging in Oncology, Kim et al (Eds)., Springer Verlag, onter alia.

[0112] Measurement of level of Ax1 polynucleotide may be determined by a method selected from: RT-PCR analysis, in-situ hybridization, polynucleotide microarray and Northern blotting. Such methods are well-known in the art, for example for in-situ hybridization Andreeff & Pinkel (Editors) (1999), "Introduction to Fluorescence In Situ Hybridization: Principles and Clinical Applications", John Wiley & Sons Inc.; and for Northern blotting Trayhurn (1996) "Northern blotting", *Proc Nutr Soc;* 55(IB): 583-9 and Shifman & Stein (1995) "A reliable and sensitive method for non-radioactive Northern blot analysis of nerve growth factor mRNA from brain tissues", *Journal of Neuroscience Methods;* 59: 205-208 inter alia.

[0113] This application is also directed to a method of diagnosing nephropathy, preferably diabetic nephropathy or kidney fibrosis, in a subject comprising determining in a sample from the subject the level of an Ax1 receptor polypeptide, wherein a higher level of the polypeptide compared to the level in a subject free of nephropathy is indicative of nephropathy. In preferred embodiments the Ax1 receptor comprises consecutive amino acids, the sequence of which is set forth in SEQ ID NO:5, SEQ ID NO:2 or SEQ ID NO:4. The sample is taken from a bodily fluid, preferably blood or urine.

[0114] The above discussion provides a factual basis for the use of the sequences of the present invention to identify nephropathy-regulated genes and provide diagnostic probes. The methods employed and the utility of the present invention are demonstrated by the following non-limiting examples.

METHODS

[0115] General Methods in Molecular Biology

[0116] Standard molecular biology techniques known in the art and not specifically described were generally followed as in Sambrook et al., *Molecular Cloning. A Laboratory Manual,* Cold Spring Harbor Laboratory Press, New York (1989), and in Ausubel et al., *Current Protocols in Molecular Biology,* John Wiley and Sons, Baltimore, Md. (1989) and in Perbal, *A Practical Guide to Molecular Cloning,* John Wiley & Sons, New York (1988), and in Watson et al., *Recombinant DNA,* Scientific American Books, New York and in Birren et al (eds) *Genome Analysis: A Laboratory Manual Series, Vols.* 1-4 Cold Spring Harbor Laboratory Press, New York (1998) and methodology as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057 and incorporated herein by reference. Polymerase chain reaction (PCR) was carried out generally as in *PCR Protocols: A Guide To Methods And Applications,* Academic Press, San Diego, Calif. (1990). In situ (In cell) PCR in combination with Flow Cytometry can be used for detection of cells containing specific DNA and mRNA sequences (Testoni et al., 1996, Blood 87:3822.)

[0117] General Methods in Immunology

[0118] Standard methods in immunology known in the art and not specifically described are generally followed as in Stites et al (eds), Basic and Clinical Immunology (8th Edition), Appleton & Lange, Norwalk, Conn. (1994) and Mishell and Shiigi (eds), Selected Methods in Cellular Immunology, W. H. Freeman and Co., New York (1980).

[0119] Immunoassays

[0120] In general ELISAs, where appropriate, are one type of immunoassay employed to assess a specimen. ELISA assays are well known to those skilled in the art. Both polyclonal and monoclonal antibodies can be used in the assays. Where appropriate other immunoassays, such as radioimmunoassays (RIA) can be used as are known to those skilled in the art. Available immunoassays are extensively described in the patent and scientific literature. See, for example, U.S. Pat. Nos. 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3;935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219; 5,011,771 and 5,281,521 as well as Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Harbor, New York, 1989.

[0121] Antibody Production

[0122] The term "antibody", as herein defined, includes monoclonal antibodies (Mabs), polyclonal antibodies and also antibody fragments, such fragments having antibody functional activity and that can be prepared from antibodies and include Fab, $F(ab')_2$, Fv and scFv prepared by methods known to those skilled in the art (Bird et al. (1988) Science 242:423-426). Antibodies may be monoclonal, polyclonal or recombinant.

[0123] Conveniently, antibodies may be prepared against the immunogen or portion thereof, for example, a synthetic peptide based on the sequence, or prepared recombinantly by cloning techniques or the natural gene product and/or portions thereof may be isolated and used as the immunogen. Immunogens can be used to produce antibodies by standard antibody production technology well known to those skilled in the art, as described generally in Harlow and Lane (1988), Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., and Borrebaeck (1992), Antibody Engineering—A Practical Guide, W. H. Freeman and Co., NY. Antibody fragments may also be prepared from the antibodies and include Fab, $F(ab')_2$, and Fv by methods known to those skilled in the art.

[0124] For producing polyclonal antibodies a host, such as a rabbit or goat, is immunized with the immunogen or immunogen fragment, generally with an adjuvant and, if necessary, coupled to a carrier; antibodies to the immunogen are collected from the sera. Further, the polyclonal antibody can be absorbed such that it is monospecific; that is, the sera can be absorbed against related immunogens so that no cross-reactive antibodies remain in the sera, rendering it monospecific.

[0125] For producing monoclonal antibodies the technique involves hyperimmunization of an appropriate donor with the immunogen, generally a mouse, and isolation of splenic antibody-producing cells. These cells are fused to an immortal cell, such as a myeloma cell, to provide a fused cell hybrid that is immortal and secretes the required antibody. The cells are then cultured, in bulk, and the monoclonal antibodies harvested from the culture media for use.

[0126] For producing recombinant antibody (see generally Huston et al. (1991) "Protein engineering of single-chain Fv analogs and fusion proteins" in Methods in Enzymology (J J Langone, ed., Academic Press, New York, N.Y.) 203:46-88; Johnson and Bird (1991) "Construction of single-chain Fvb derivatives of monoclonal antibodies and their production in *Escherichia coli* in Methods in Enzymology (J J Langone, ed.; Academic Press, New York, NY) 203:88-99; Memaugh and Memaugh (1995) "An overview of phage-displayed recombinant antibodies" in Molecular Methods In Plant Pathology (R P Singh and U S Singh, eds.; CRC Press Inc., Boca Raton, Fla.:359-365), messenger RNAs from antibody-producing B-lymphocytes of animals, or hybridoma are reverse-transcribed to obtain complementary DNAs (cDNAs). Antibody cDNA, which can be full or partial length" is amplified and cloned into a phage or a plasmid. The cDNA can be a partial length of heavy and light chain cDNA, separated or connected by a linker. The antibody, or antibody fragment, is expressed using a suitable expression system to obtain recombinant antibody. Antibody cDNA can also be obtained by screening pertinent expression libraries.

[0127] The antibody can be bound to a solid support substrate or conjugated with a detectable moiety or be both bound and conjugated as is well known in the art. (For a general discussion of conjugation of fluorescent or enzymatic moieties see Johnstone & Thorpe (1982.), Immunochemistry in Practice, Blackwell Scientific Publications, Oxford). The binding of antibodies to a solid support substrate is also well known in the art (for a general discussion, see Harlow & Lane (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Publications, New York; and Borrebaeck (1992), Antibody Engineering—A Practical Guide, W. H. Freeman and Co.). The detectable moieties contemplated with the present invention can include, but are not limited to, fluorescent, metallic, enzymatic and radioactive markers such as biotin, gold, ferritin, alkaline phosphatase, β-galactosidase, peroxidase, urease, fluorescein, rhodamine, tritium, ^{14}C and iodination.

[0128] Recombinant Protein Purification

[0129] For standard purification, See Marshak et al. (1996), "Strategies for Protein Purification and Characterization. A laboratory course manual." CSHL Press. Specific purification protocols used for the production of Ax1 protein are described in the examples part.

[0130] Transgenic and Knockout Methods

[0131] The present invention provides for a transgenic gene and a polymorphic gene animal and cellular (cell line) model, as well as for a knockout model. These models are constructed using standard methods known in the art and as set forth in U.S. Pat. Nos. 5,487,992; 5,464,764; 5,387,742; 5,360,735; 5,347,075; 5,298,422; 5,288,846; 5,221,778; 5,175,385; 5,175,384; 5,175,383; 4,736,866; as well as Burke and Olson (1991) "Preparation of Clone Libraries in Yeast Artificial-Chromosome Vectors" in Methods in Enzymology, 194, "Guide to Yeast Genetics and Molecular Biology", eds. C. Guthrie and G. Fink, Academic Press, Inc., Chap. 17:251-270; Capecchi (1989) "Altering the genome by homologous recombination", Science, 244:1288-1292; Davies et al. (1992) "Targeted alterations in yeast artificial chromosomes for inter-species gene transfer", Nucleic Acids Research, 20 (11): 2693-2698; Dickinson et al. (1993) "High frequency gene targeting using insertional vectors", Human Molecular Genetics, 2(8):1299-1302; Duff and Lincoln (1995) "Insertion of a pathogenic mutation into a yeast artificial chromosome containing the human APP gene and expression in ES cells", Research Advances in Alzheimer's Disease and Related Disorders Khalid Iqbal (Editor), James A. Mortimer (Editor), Bengt Winblad (Editor), Henry M. Wisniewski (Editor); Huxley et al. (1991) "The human HPRT gene on a yeast artificial chromosome is functional when transferred to mouse cells by cell fusion", Genomics, 9:742-750; Jakobovits et al. (1993) "Germ-line transmission and expression of a human-derived yeast artificial chromosome", *Nature,* 362: 255-261; Lamb et al. (1993) "Introduction and expression of the 400 kilobase precursor amyloid protein gene in transgenic mice", Nature Genetics, 5:22-29; Pearson and Choi (1993) Expression of the human b-amyloid precursor protein gene from a yeast artificial chromosome in transgenic mice. Proc. Natl. Acad. Sci. (USA), 90:10578-10582; Rothstein, (1991) "Targeting, disruption, replacement, and allele rescue: integrative DNA transformation in yeast" in Methods in Enzymology, 194, "Guide to Yeast Genetics and Molecular Biology", eds. C. Guthrie and G. Fink, Academic Press, Inc., NY, Chap. 19:281-301; Schedl et al. (1993) "A yeast artificial chromosome covering the tyrosinase gene confers copy number-dependent expression in transgenic mice", Nature, 362:258-261; Strauss et al. (1993) "Germ line transmission of a yeast artificial chromosome spanning the murine a_1 (I) collagen locus", Science, 259:1904-1907. Further, PCT patent applications WO 94/23049, WO 93/14200, WO 94/06908, WO 94/28123 also provide information.

[0132] Further one parent strain, instead of carrying a direct human transgene, may have the homologous endogenous gene modified by gene targeting such that it approximates the transgene. That is, the endogenous gene has been "humanized" and/or mutated (Reaume et al. (1996) J Biol Chem. 271(38):23380-23388.). It should be noted that if the animal and human sequences are essentially homologous, a "humanized" gene is not required. The transgenic parent can also carry an overexpressed sequence, either the non-mutant or a mutant sequence and humanized or not as required. Herein, the term "transgene" is therefore used to refer to all these possibilities.

[0133] Additionally, cells can be isolated from the offspring that carry a transgene from each transgenic parent and that are used to establish primary cell cultures or cell lines as is known in the art.

[0134] Where appropriate, a parent strain will be homozygous for the transgene. Additionally, where appropriate, the endogenous non-transgene in the genome that is homologous to the transgene will be non-expressive. Herein, by the term "non-expressive" is meant that the endogenous gene will not be expressed and that this non-expression is heritable in the offspring. For example, the endogenous homologous gene could be "knocked-out" by methods known in the art. Alternatively, the parental strain that receives one of the transgenes could carry a mutation at the endogenous homologous gene rendering it non-expressed.

[0135] Gene Therapy

[0136] "Gene therapy" as used herein refers to the transfer of genetic material (e.g., DNA or RNA) of interest into a host to treat or prevent a genetic or acquired disease or condition phenotype. The genetic material of interest encodes a product (e.g., a protein, polypeptide, peptide, functional RNA, AS) the production of which is desired in vivo. In particular, the use of antisense molecules (anti -Ax1 polynucleotide) in gene therapy may be used in accordance with the anti fibrosis aspect of the invention.

[0137] Gene therapy of the present invention can be carried out in vivo or ex vivo. Ex vivo gene therapy requires the isolation and purification of patient cells, the introduction of a therapeutic gene and the introduction of the genetically altered cells back into the patient. A replication-deficient virus such as a modified retrovirus can be used to introduce the therapeutic gene into such cells. For example, mouse Moloney leukemia virus (MMLV) is a well-known vector in clinical gene therapy trials. See, e.g., Boris-Lauerie et al., Curr. Opin. Genet. Dev., 3, 102-109 (1993).

[0138] In contrast, in vivo gene therapy does not require isolation and purification of a patient's cells. The therapeutic gene is typically "packaged" for administration to a patient such as in liposomes or in a replication-deficient virus such as adenovirus as described by Berkner, K. L., in Curr. Top. Microbiol. Immunol., 158, 39-66 (1992) or adeno-associated virus (AAV) vectors as described by Muzyczka, N., in Curr. Top. Microbiol. Immunol., 158, 97-129 (1992) and U.S. Pat. No. 5,252,479. In an alternative embodiment, if the host gene is defective, the gene is repaired in situ (Culver (1998) "Site-Directed recombination for repair of mutations in the human ADA gene" (Abstract) Antisense DNA & RNA based therapeutics, Coronado, CA.). Another approach is administration of "naked DNA" in which the therapeutic gene is directly injected into the bloodstream or muscle tissue, for example wherein the therapeutic gene is introduced into the target tissue by microparticle bombardment using gold particles coated with the DNA. Gene therapy vectors can be delivered to a subject by methods known in the art, for example, intravenous injection, local administration (see U.S. Pat. No. 5,328,470) or by stereotactic injection (see e.g., Chen et al. (1994) PNAS 91:3054-3057),

and as generally described in Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Harbor Laboratory, New York (1989, 1992); in Ausubel et al., Current Protocols in Molecular Biology, John Wiley and Sons, Baltimore, Maryland (1989); in Chang et al., Somatic Gene Therapy, CRC Press, Ann Arbor, Mich. (1995); in Vega et al., Gene Targeting, CRC Press, Ann Arbor, Mich. (1995); Vectors: A Survey of Molecular Cloning Vectors and Their Uses, Butterworths, Boston Mass. (1988); and Gilboa, E et al. (1986) Transfer and expression of cloned genes using retroviral vectors. BioTechniques 4(6):504-512; these vectors may include, for example, stable or transient transfection, lipofection, electroporation and infection with recombinant viral vectors. In addition, see United States Patent 4,866,042 for vectors involving the central nervous system and also U.S. Pat. Nos. 5,464,764 and 5,487,992 for positive-negative selection methods.

[0139] The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, e.g. retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

[0140] Cell types useful for gene therapy of the present invention include lymphocytes, hepatocytes, myoblasts, fibroblasts, and any cell of the eye such as retinal cells, epithelial and endothelial 20 cells. Preferably the cells are T lymphocytes drawn from the patient to be treated, hepatocytes, any cell of the eye or respiratory or pulmonary epithelial cells. Transfection of pulmonary epithelial cells can occur via inhalation of a neubulized preparation of DNA vectors in liposomes, DNA-protein complexes or replication-deficient adenoviruses. See, e.g., U.S. Pat. No. 5,240,846. For a review of the subject of gene therapy, in general, see the text "*Gene Therapy",* August et al. Advances in Pharmacology 40, Academic Press, 1997.

[0141] Delivery of Gene Products/Therapeutics (Compound)

[0142] The compound of the present invention is administered and dosed in accordance with good medical practice, taking into account the clinical condition of the individual patient, the site and method of administration, scheduling of administration, patient age, sex, body weight and other factors known to medical practitioners. The pharmaceutically "effective amount" for purposes herein is thus determined by such considerations as are known in the art. The amount must be effective to achieve improvement including, but not limited to, improved survival rate or more rapid recovery, or improvement or elimination of symptoms and other indicators as are selected as appropriate measures by those skilled in the art.

[0143] In the method of the present invention, the compound of the present invention can be administered in various ways. It should be noted that it can be administered as the compound or as a pharmaceutically acceptable salt and can be administered alone or as an active ingredient in combination with pharmaceutically acceptable carriers, diluents, adjuvants and vehicles. The compounds can be administered orally, subcutaneously or parenterally including intravenous, intraarterial, intramuscular, intraperitoneally, and intranasal administration, as well as intrathecal and infusion techniques. Implants of the compounds are also useful. The patient being treated is a warm-blooded animal and, in particular, mammals including man. The pharmaceutically acceptable carriers, diluents, adjuvants and vehicles as well as implant carriers generally refer to inert, non-toxic solid or liquid fillers, diluents or encapsulating material not reacting with the active ingredients of the invention.

[0144] It is noted that humans are treated generally longer than the mice or other experimental animals exemplified herein, which treatment has a length proportional to the length of the disease process and drug effectiveness. The doses may be single doses or multiple doses over a period of several days, but single doses are preferred.

[0145] When administering the compound of the present invention parenterally, it will generally be formulated in a unit dosage injectable form (e.g., solution, suspension, emulsion). The pharmaceutical formulations suitable for injection include sterile aqueous solutions or dispersions and sterile powders for reconstitution into sterile injectable solutions or dispersions. The carrier can be a solvent or dispersing medium containing, for example, water, ethanol, polyol (e.g., glycerol, propylene glycol, liquid polyethylene glycol), suitable mixtures thereof, and vegetable oils.

[0146] Proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Non-aqueous vehicles such a cottonseed oil, sesame oil, olive oil, soybean oil, corn oil, sunflower oil, or peanut oil and esters, such as isopropyl myristate, may also be used as solvent systems for compound compositions. Additionally, various additives which enhance the stability, sterility, and isotonicity of the compositions, including antimicrobial preservatives, antioxidants, chelating agents, and buffers, can be added. Prevention of the action of microorganisms can be ensured by various antibacterial and antifungal agents, e.g., parabens, chlorobutanol, phenol and sorbic acid. In many cases, it will be desirable to include isotonic agents, for example, sugars, sodium chloride, and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, for example, aluminum monostearate and gelatin. According to the present invention, however, any vehicle, diluent, or additive used would have to be compatible with the compounds.

[0147] Sterile injectable solutions can be prepared by incorporating the compounds utilized in practicing the present invention in the required amount of the appropriate solvent with various of the other ingredients, as desired.

[0148] A pharmacological formulation of the present invention can be administered to the patient in an injectable formulation containing any compatible carrier, such as various vehicles, adjuvants, additives, and diluents; or the compounds utilized in the present invention can be administered parenterally to the patient in the form of slow-release subcutaneous implants or targeted delivery systems such as monoclonal antibodies, vectored delivery, iontophoretic, polymer matrices, liposomes, and microspheres. Examples of delivery systems useful in the present invention include those presented in U.S. Pat. Nos: 5,225,182; 5,169,383; 5,167,616; 4,959,217; 4,925,678; 4,487,603; 4,486,194;

4,447,233; 4,447,224; 4,439,196 and 4,475,196. Other such implants, delivery systems, and modules are well known to those skilled in the art.

[0149] A pharmacological formulation of the compound utilized in the present invention can be administered orally to the patient. Conventional methods such as administering the compounds in tablets, suspensions, solutions, emulsions, capsules, powders, syrups and the like are usable. Known techniques that deliver the compound orally or intravenously and retain the biological activity are preferred.

[0150] In one embodiment, the compound of the present invention can be administered initially by intravenous injection to bring blood levels to a suitable level. The patient's blood levels are then maintained by an oral dosage form, although other forms of administration, dependent upon the patient's condition and as indicated above, can be used. The quantity to be administered will vary for the patient being treated and will vary from about 100 ng/kg of body weight to 100 mg/kg of body weight per day and preferably will be from 10 g/kg to 10 mg/kg

[0151] Throughout this application, various publications are referenced by author and year and patents, including United States Patents, are referenced by number. . The disclosures of these publications and patents in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

[0152] The invention has been described in an illustrative manner, and it is to be understood that the terminology that has been used is intended to be construed in the nature of description rather than of limitation.

[0153] Obviously, many modifications and variations of the present invention are possible in light of the above teachings. It is therefore to be understood that within the scope of the described invention, the invention may be practiced otherwise than as specifically described.

EXAMPLE 1

Identification of Ax1 Overexpression by Microarray Hybridization Study

[0154] In accordance with the present invention, the microarray hybridization approach was utilized in order to discover genes that are differentially regulated in diabetic nephropathy and kidney fibrosis.

[0155] Microarray-based analysis of gene expression was based on the analysis of human fibroblasts subject to selected stimuli resulting in changes in extracellular collagen accumulation and proliferation—the hallmarks of fibrosis. According to the present invention, a specific "Fibrosis" DNA chip was first prepared followed by a microarray hybridization experiments with 19 different types of probes. Analysis of the results was carried out by proprietary algorithms, and analysis of the selected set of genes was performed by using bioinformatics and the scienctific literature.

[0156] Perparation of Specific "Fibrosis" DNA Chip

[0157] A dedicated human "Fibrosis" DNA chip was prepared according to assignee's SDGI method (PCT Application Publication No. WO 01/75180) from growth-arrested human fibroblasts. Growth arrest was imposed by the treatments presented in Table 1 below:

TABLE 1

	Biological material for "Fibrosis" chip preparation Treatment
1	G1 arrested serum-starved 1.p. HFs*
2	1.p. HFs* 36 hr and 48 hr following 8Gy γ-irradiation
3	1.p. HFs* 5 days after addition of H_2O_2 200 μM
4	1.p. HFs* following UV (growth-arresting dose)
5	1.p. HFs* 48 hr following Bleomycin treatment 50 ng/ml
6	1.p. HFs* 48 hr following Etoposide treatment 400 ng/ml
7	1.p. HFs* 48 hr following Adriamycin treatment 50 ng/ml
8	Senescent HFs from normal individuals
9	Senescent HFs from individuals with Werner syndrome
10	Senescent HFs from individuals with Progeria

1.p. HF*—low passage human fibroblasts

[0158] Unless indicated otherwise, all human fibroblasts (HFs) were at passage 15 prior to treatment. RNA from all treated HFs was prepared, pooled and used for library preparation by the proprietary SDGI method of the assignee. This chip also contained human ESTs coding for genes known to play a part in apoptosis, cytotoxicity and replicative cellular senescence.

[0159] Fibroblast Cultivation

[0160] Normal human fetal lung fibroblasts (WI-38, Coriell Cell Repositories) were cultured and sub-cultured in DMEM, supplemented with 10% inactivated fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin. Fibroblasts were grown to confluence in 25 cm^2 tissue flasks and sub-cultured after trypsinization (0.5% trypsin-EDTA in Hank's balanced solution without Ca^{2+} and Mg^{2+}) at 37° C. in an atmosphere of 5% CO_2. Two ml of trypsin were added to each flask and incubated for 5 min; then cultures were centrifuged (5 min, 1000 rpm) and fresh medium was added to the pellet. Splitting conditions were 1:4-1:6.

[0161] Since the hallmarks of fibrotic disease are fibroblast proliferation and/or enhanced synthesis of extracellular matrix components (mainly collagen), different treatment regimes were used and the rates of both proliferation and collagen synthesis by the treated fibroblasts cultured in vitro was examined.

[0162] Fibroblast Proliferation Assay

[0163] The proliferation rate of sub-confluent fibroblasts was evaluated by staining with neutral red (BioRad). Fibroblasts were seeded in 96-well plate (6×10^3/well) in 200 μl of supplemented DMEM/10% FBS. After overnight culture, wells were washed twice with supplemented DMEM/20% FBS. Then, either TGF-β (2-20 ng/ml) or deferoxamine mesylate (DFO, which leads to conditions of chemical hypoxia) at a concentration of 100 mM was added in 200 μl of supplemented DMEM/2% FBS for either 16 hours, 24 hours, 72 hours, or 5 days.

[0164] In the case of glucose treatments, after overnight culture, cell-containing wells were washed twice with supplemented glucose-free DMEM/2% FBS. Working concentrations of glucose (5.5 mM, 15 mM, 27.5 mM, or 55 mM) were prepared by dissolving stock solution (110 mM)

in supplemented DMEM without glucose/2% FBS. Prepared solutions of glucose were added to fibroblast cultures for either 24 or 72 hours.

[0165] Upon completion of incubation, cells were stained with 100 μl of 1% neutral red for 2 hours. After washing with cold PBS, fibroblast monolayers were fixed with 200 μl of ethanol-Sorenson buffer solution (1:1) for 10 min. Optical density was measured with an automated spectrophotometer (λ=540 nm).

[0166] Collagen Production Assay

[0167] Collagen production by confluent fibroblast monolayers was assessed by [^{3}H]-proline incorporation into collagenous proteins. Fibroblasts were seeded in 24-well tissue culture plates (2×10^4/well) and grown in 1 ml of supplemented DMEM/10%FBS until confluence.

[0168] Confluent fibroblast cultures were incubated with prepared solutions for either 24 or 48 hr. Then [^{3}H]-proline (10 μCi/well) was added and cultures were incubated for an additional 24 hr. At the end of the incubation, medium was decanted and incubated with or without collagenase for 18 hr, followed by precipitation with 50% and 10% TCA. The production of collagen was determined as the difference between total [^{3}H]proline-containing proteins in the sample incubated without collagenase and those left after collagenase digestion. To determine the number of cells in each well, fibroblasts were detached by trypsinization on the last day of the experiment, and counted in a hemocytometer.

[0169] Probes for microarray hybridization were derived from these treated fibroblasts. In accordance with the present invention, treatments that are relevant for diabetic nephropathy development were used, such as glucose deprivation or hypoxia (modeling ischemic conditions that develop in fibrotic kidney); high glucose (modeling diabetic hyperglycemia) and TGF-β induction (modeling a fibrotic condition that is characterized by growth factor and cytokine imbalance).

[0170] More specifically, human fibroblasts were treated as followed:

[0171] 1. glucose at 4 different concentrations (5.5, 15, 27.5, or 55 mM) for 24 and 72 hr

[0172] 2. TGF-β at 2-20 ng/ml, for 24 or 72 hr

[0173] 3. DFO deferoxamine at a concentration of 100 mM, dissolved in 0.5 ml of DMEM, containing 5% FCS, 50 μg/ml β-aminoproprionitrile, and 50 μg/ml ascorbic acid (modified DMEM). For 24, 48 and 72 hours.

[0174] The analysis of proliferation rate of these cultured fibroblasts showed that cultivation of fibroblasts for 24 hrs in glucose-free medium and in 55 mM glucose resulted in a decrease of their proliferation rate by 20% and 30%, respectively, compared to control cultures. Addition of glucose at different concentrations (from 5.5 mM to 27.5 mM) practically did not affect fibroblast proliferation compared to the control. A significant decrease in fibroblast proliferation was observed after addition of DFO (from 20% decrease after 16 hr incubation to 80% decrease after 5 days of treatment). TGF-β, added at concentrations of 2 and 20 ng/ml, led to an increase in the fibroblast proliferation rate by ~60% after 24 hrs treatment.

[0175] As for collagen synthesis rate, all treatments (except for 55 mM glucose) led to increased collagen production by fibroblasts. The most significant effect was observed after addition of TGF-β at concentrations of 2-20 ng/ml, providing enhancement in collagen production by 110-180%.

[0176] In the next step, the RNA from these treated fibroblasts was extracted and used for preparation of probes for microarray hybridization. The scheme of hybridization is presented below:

TABLE 2

Hybridization scheme

Probe name	Dye	PROBE 1	Probe name	Dye	PROBE 2
FG1A	Cy3	Untreated human fibroblasts-Common Normalizing Probe	FG1B	CyS	1.p. untreated HFs*
FG19A			FG19B		1.p. untreated HFs*
FG18A			FG18B		1.p. HFs* w/o glucose 72 hr
FG17A			FG17B		1.p. HFs* TGP-β 20 ng/μl 72 h
FG16A			FG16B		1.p. HFs* TGF-β 20 ng/μl 24 h
FG15A			FG15B		1.p. HFs* w/o glucose 24 h
FG14A			FG14B		1.p. HFs* TGF-β 2 ng/μ 72 h
FG13A			FG13B		1.p. HFs* TGF-β 2 ng/ml 24 h
FG12A			FG12B		1.p. HFs* 55 mM glucose 72 h
FG11A			FG11B		1.p. HFs* 5.5 mM glucose 24 h,
FG10A			FG10B		1.p. HFs* Hypoxia 5 days
FG9A			FG9B		1.p. HFs* 55 mM glucose 72 h
FG8A			FG8B		1.p. HFs* 55 mM Glucose 24 h
FG7A			FG7B		1.p. HFs* Hypoxia 3 days
FG6A			FG6B		1.p. HFs* 275 mM Glucose 72 h
FG5A			FG5B		1.p. HFs* 27.5 mM glucose 24 h
FG4A			FG4B		1.p. HFs* hypoxia 16 h
FG3A			FG3B		1.p. HFs* 15 mM glucose 72 h
FG2A			FG2B		1.p. HFs* 15 mM glucose 24 h

1.p. HFs*- low passage human fibroblasts

[0177] Probe 1 was identical in all hybridization experiments, and was produced with RNA extracted from untreated human fibroblasts (passage 15). This probe served both as a biological control and as a common normalizing probe that allowed comparison of results obtained from different hybridization experiments.

[0178] In accordance with the present invention, a total of 19 hybridization experiments were performed. In two hybridization experiments (FG1 and FG19), the common normalizing probe (Probe I in all hybridization experiments) was hybridized against itself (i.e., Probe 1 was identical to Probe 2). In general, these hybridization experiments were conducted in order to determine labeling quality and to evaluate the ability of the common normalizing probe to detect most of the cDNA clones printed on the chip.

[0179] Bioinformatics Analysis of Gene Expression Results

[0180] The proprietary statistical analysis of the assignee of microarray hybridization results is based on the assumption that changes in gene expression correlate with different physiological and pathological conditions and, in many instances, underlie them. Thus, in a given set of experiments, a certain treatment regime/condition is associated with a particular gene expression profile. Furthermore, we assume that some hierarchy exists among the different (patho) physiological conditions/treatments, i.e., some are more similar than others.

[0181] The final goal of such an analysis is to elucidate both specific and general mechanisms underlying complex biological phenomena by comparison of gene expression patterns within a large panel of conditions, each representing some of its aspects. More specifically, in the set of hybridization results generated in accordance with the present invention, we anticipated observing groups of genes that their expression was either common or unique to different types of conditions relevant to diabetic nephropathy (hypoxia, high glucose, TGF-β), and wherein the response to the applied treatment was either acute or chronic.

[0182] Results of Hybridization Analysis

[0183] In accordance with the present invention, in human fibroblasts differentially treated in vitro, a set of 46 genes was identified, the activity of which was significantly up-regulated by various types of applied treatments.

[0184] The identified gene products fell into nine distinct functional groups:

[0185] 1. Extracellular matrix proteins and receptors to extracellular matrix proteins;

[0186] 2. Secreted growth factor interacting proteins and potential growth factor receptors;

[0187] 3. Signal transduction adaptor proteins;

[0188] 4. Cytoskeletal proteins (mostly related to actin cytoskeleton function);

[0189] 5. Ca^{2+}-binding proteins;

[0190] 6. ER-resident proteins;

[0191] 7. Nuclear import mediators;

[0192] 8. Proteins involved in RNA and protein synthesis and processing;

[0193] 9. Novel genes

[0194] The 46 up-regulated genes identified were divided as follows:

[0195] (a) 11 were known genes with known functions with recognized involvement in fibrosis (collagens type III and I (α1 and α2), fibronectin, decorin, β-ig-h3, integrin, TIMP3, CD44, smooth muscle actin, and Arp2/3 (Arc34);

[0196] (b) 28 were known genes with known function but with previously unknown involvement in fibrosis. Ax1, the subject of the present invention, falls into this category;

[0197] (c) 2 were genes coding for proteins with unknown function and unknown involvement in fibrosis, and

[0198] (d) 5 were novel genes.

[0199] Using the microarray hybridizaton technique it was found that the expression of Ax1 has been induced by TGF-β treatment of human fibroblasts by at least 2 fold.

EXAMPLE 2

Validation of Ax1 as a TGF-β Induced Gene (Expression and Phosphorylation Status) by in vitro Experiments

[0200] In order to verify the chip hybridization results, the response of endogenous Ax1 expression to TGF-β stimulation was monitored by Western blot analysis. Total cellular proteins from various cell lines, (of which Rat1 cell line was also stimulated by TGF-β (5 ng/ml for 24 hr) were extracted, and the expression of Ax1 was analyzed by Western blot analysis. Thirty (30) μg of total cellular lysate were run on an 8% SDS gel.

[0201] Results showed slight up-regulation following TGF-β stimulation in Rat1 cells (shown in **FIG. 1**).

[0202] Further experiments were done on Rat1 cells that were serum starved for 24 hr and then stimulated for the indicated time (15 min-2 hr) with 5 ng/ml TGF-β.

[0203] Results show that indeed TGF-β induces Ax1 protein level (**FIG. 2**), following 15 min of TGF-β treatment. Increase in its phosphorylation is also observed (**FIG. 3**) suggesting than in response to TGF-β, Ax1 protein is induced and functionally activated.

EXAMPLE 3

Assessment of in vivo Models for Kidney Fibrosis by Morphology, Immunostaining and In situ Hybridization

[0204] Morphology

[0205] To assess general morphology, paraffin kidney sections were stained by hematoxilin-eosin (HE). The Sirius Red (SR) staining was used to reveal collagen in the sections.

[0206] Immunostaining

[0207] Accumulation of interstitial myofibroblasts is regarded as an important initial step in the development of the renal fibrotic process. To reveal myofibroblasts, monoclonal antibody specific to α-smooth muscle actin (clone

1A4) was used for the peroxidase-antiperoxidase (PAP) immunostaining of kidney paraffin sections. The monoclonal antibody PC-10 was used for the immmunostaining of proliferating cell nuclear antigen (PCNA). To achieve adequate PCNA immunostaining, de-paraffinized sections were subjected to antigen retrieval procedure before performing PAP staining.

[0208] In situ Hybridization

[0209] ^{35}S-labeled riboprobes were synthesized and hybridized to kidney paraffin sections according to standard protocol. After the post-hybridization washing step, sections were air-dried and macro-autoradiography was performed by exposing the slides to X-ray film overnight. For micro-autoradiography, slides were dipped into nuclear track emulsion and stored in darkness at 4° C. Exposed slides were developed after 2-3 weeks and sections were slightly counter-stained with HE and cover-slipped for microscopic examination.

[0210] Probes for in situ Hybridization

[0211] The cDNAs used as the templates for riboprobe synthesis were rat osteopontin cDNA, mouse transforming growth factor β1 cDNA, mouse procollagen α1(I) cDNA and mouse thrombosponding cDNA.

[0212] Results:

[0213] ZDF Rats

[0214] Samples of 9-month-old ZDF rats (zucker diabetic fatty rats) presented hydronephrotic kidneys with dilated calyces. Microscopically these samples presented a picture of glomerulosclerosis and tubulointerstitial fibrosis. In accordance with these morphological changes, the expression of marker genes as measured by in situ hybridization (osteopontin (OPN), transforming growth factor β1 (TGF-β1) and procollagen α1(l) (Coll)) was significantly changed when compared to normal kidneys. Strong OPN expression was detectable in all tubular structures in both cortex and medulla. The TGF-β1 expression was widespread throughout interstitial cells. Some epithelial cells also showed TGF-β1 expression. Coll expression was detectable by in situ hybridization in most interstitial cells within the medulla, while cortical expression was “focal”.

[0215] Aged fa/fa (Obese Zucker) Rats

[0216] Samples of 12-month-old fa/fa rats presented strong glomerulosclerosis and diffuse tubulointerstitial fibrosis throughout the cortex and the medulla. The pattern of marker gene expression corresponded to morphological changes. OPN was expressed by tubular structures in the cortex and the medulla. Multiple interstitial cells expressed TGF-β1. Significantly, multiple foci and single interstitial cells showed strong Coll expression in both cortex and medulla so that the number of Coll -expressing cells appeared to be higher in fa/fa samples than in ZDF samples.

[0217] Interestingly, Coll expression was not detected in glomeruli of either ZDF or fa/fa rats in spite of the prominent accumulation of collagen, as revealed by Sirius Red staining. This suggested a low steady state level of Coll mRNA in glomerular cells.

[0218] Aged SD (Normal) Rats

[0219] Samples of aged SD rats showed increased accumulation of collagen in glomeruli and interstitial space and increased expression of the marker genes. Significantly, the intensity of fibrotic change varied among samples so that one of four samples studied displayed very few changes compared with young animals; fibrotic change in another sample was confined to “polar” regions, and two samples showed uniform accumulation of collagen and elevated expression of marker genes throughout the sections.

[0220] Goto Kakizaki (GK)/Wistar (Normal) 48-week-old Rats

[0221] Samples of both GK and Wistar 48-week-old rats showed an accumulation of collagen in glomeruli and interstitial space. This accumulation was more pronounced in the GK samples. Two samples were used for mRNA isolation: C9 and GK9. Both were hybridized to the probe specific for IGFBP4. The in situ hybridization results showed that the GK sample demonstrated elevated expression of this gene.

[0222] Permanent UUO.

[0223] A known model for fibrosis was employed-unilateral urether occlusion (UUO) . One of the urethers was occluded (see below) and animals were sacrifized 1,5,10, 15,20 and 25 days following occlusion.

[0224] Permanent UUO resulted in rapid activation (5 days of UUO) of collagen synthesis by interstitial cells in both medulla and cortex. By 20-25 days of UUO, significant amounts of interstitial collagen were deposited in the interstitial space while glomerular accumulation of collagen was confined to the outer capsule. Thus, permanent UUO samples provided an acute model of tubulointerstitial renal fibrosis without prominent glomeruloselerotic changes.

[0225] The above models can be used as model systems for testing the therapeutic efficacy of inhibitors identified via any of the screening systems described

EXAMPLE 4

Protocol for Permanent Unilateral Ureteral Obstruction (UUO)

[0226] Test System

[0227] Strain: Male Sprague-Dawley rats (9 weeks of age)

[0228] Group Size: n=5 for operated rat; n=3 for sham-operated rats

[0229] Number of groups: 6 for both sham-operated and operated (i.e., 1 day, 5 days, 10 days, 15 days, 20 days and 25 days post-operation or post-sham operation)

[0230] Procedure

[0231] Rats were anaesthetized with Ketamin/Xylazine and the abdominal cavity was opened. After being exposed, the ureter from the right kidney was ligated with a suture over it (UUO). In sham-operated rats, the ureter was exposed but not ligated.

[0232] Study Termination

[0233] The study was terminated 24 hr, 5 days, 10 days, 15 days, 20 days and 25 days after the UUO procedure or after the sham operation. At this time point, the rats were sacri-

ficed by exsanguination under CO_2 asphyxiation in order to collect the right kidney. After the capsule was removed the kidney was cut transversely. Half was fixed in 10% buffered formalin and the other half was immediately transferred to an eppendorf tube and frozen in liquid nitrogen for RNA analysis.

EXAMPLE 5

Analysis of Expression of the Ax1 Gene in Normal and Fibrotic Human Kidneys

[0234] The expression patterns of Ax1 were studied by in situ hybridization using sections from human renal tissue samples. The samples analyzed in this pilot study included:

[0235] 1. normal human kidney (32 year old female);

[0236] 2. diabetic human kidney showing signs of glomerulosclerosis and tubulointerstitial fibrosis (62 year old male);

[0237] 3. renal sclerosis accompanied by vast diffuse fibrosis (56 year old female);

[0238] 4. rejected kidney transplant showing vascular sclerosis, lymphocyte infiltration, glomerulosclerosis and scarring fibrosis (44 year old female; 2 years after transplantation).

[0239] Representative sections of all samples were subjected to trial hybridization to the probe specific to elongation factor 1α mRNA in order to ensure the presence of hybridizable mRNA and to establish the optimal regime of pre-hybridization treatment.

[0240] The results show that in normal kidneys the expression of Ax1 is very low. On the other hand in fibrotic kindeys staining indicating higher levels of Ax1 gene in tubular epithelial cells in fibrotic regions within the kidney was observed.

[0241] Therefore, these experiments involving iii situ hybridization with human fibrotic samples suggested the involvement of the Ax1 gene in the proliferation of tubular epithelial cells in fibrotic regions within the kidney.

EXAMPLE 6

Analysis of Expression of the Ax1 Gene in Normal and Fibrotic Rat Kidney Samples

[0242] A mouse EST clone (Accession Number: BG293435 gi: 4502194) was used as the template 20 for preparation of a riboprobe complementary to rodent Ax1. The radioactively labeled probe was hybridized to the following sections:

[0243] 1. Permanent UUO multiblock comprised of control sample fixed 25 days after sham-operation; and samples fixed at 24 hr, 5 d, 10 d and 25 d of UUO (one sample per time point);

[0244] 2. Rat chronic renal failure sample: kidney of 2 year, 7 month-old rat;

[0245] 3. ZDF samples: samples of 4.5 (non-fibrotic) and 9 month-old (strongly fibrotic) ZDF kidneys;

[0246] 4. Fa/fa samples: samples of 3, 6 (non-fibrotic) and 12 month-old (strongly fibrotic) fa/fa kidneys;

[0247] 5. Rat tissue multiblock.

[0248] Analysis of in situ hybridization results demonstrated a low level of Ax1 expression in non-fibrotic samples (sham-operated UUO sample and young ZDF and fa/fa samples). Weak hybridization signal in these samples was localized to glomeruli and single interstitial/perivascular cells. Interestingly, small foci of expression in tubular epithelial cells were observed in young samples of ZDF and fa/fa kidneys. These foci were associated with small accumulations of infiltrating lymphocytes and/or interstitial cells. These latter cell types also showed hybridization signal.

[0249] Ureter obstruction resulted in prominent changes in the intensity and pattern of Ax1 hybridization signals so that after 24hr of UUO, the hybridization signal could be seen above the epithelial lining of thick ascending limbs of Henle's loop and collecting ducts in the outer medulla. The hybridization signal also spread into the cortex where collecting ducts, collecting tubules and distal tubules showed prominent hybridization signal. This pattern of expression suggested rapid activation of Ax1 transcription in the distal part of the nephron in response to obstruction. This pattern of epithelial expression was preserved throughout later time points of UUO. In addition to the epithelial signal, some accumulation of expressing cells could be seen in interstitial cells, beginning at 5 days of UUO. At least some of these interstitial cells could be identified as endothelial.

[0250] Samples representing chronic fibrotic models also showed significant changes in the pattern of Ax1 expression. Thus, aged fa/fa samples showed multiple foci of strong Ax1 expression throughout the section. Morphologically, these foci showed prominent signs of tubulointerstitial fibrosis, e.g., accumulation of interstitial cells and proliferation of the tubular epithelium. Both epithelial and interstitial cells displayed hybridization signals. A similar pattern was displayed by the aged ZDF sample. It is noteworthy that multiple foci of tubulointerstitial expression contained tubular profiles with clear signs of atrophy. Atrophic cells showed a hybridization signal for Ax1. The aged ZDF sample was prominent for the presence of areas of inflammatory infiltration. Some of the infiltrating cells showed hybridization signals for Ax1. This feature of Ax1 was observed in 4 out of 7 human fibrotic kidney samples. The Ax1-specific hybridization signal was widespread throughout the section of chronic renal failure sample (2 year, 7 month old rat). As in the rest of the fibrotic samples, expressing structures included atrophic and "proliferating", interstitial and inflammatory cells.

[0251] Thus, results of in situ hybridization studies of the Ax1 gene in rat kidney samples demonstrated a low level of expression in non-fibrotic renal tissue. Pathological samples showed expression of this gene in tubular epithelium and in some interstitial, vascular and inflammatory cells. The pattern of pathological expression was very similar to that found earlier in human fibrotic kidneys. This suggested involvement of the Ax1 gene product in the pathological mechanism common to human and rat renal fibrosis. Significantly, results of the animal study clearly demonstrated that activation of the Ax1 gene followed rapidly after the pro-fibrotic insult (UUO) and persisted at more advanced stages of the process. This suggested that the therapeutic approach aimed at the Ax1 gene product might be applicable at any stage of chronic renal failure. Moreover, rapid activation of Ax1 expression in response to UUO suggested involvement of Ax1 in acute renal failure (this suggestion

can be easily tested by in situ hybridization studies of samples obtained from patients with acute renal failure). If so, Ax1-targeted therapy may be beneficial for acute renal failure.

[0252] Multiblock analysis shows a rather widespread hybridization signal throughout rat tissues. The hybridization signal is clearly seen in the lamina propria of all compartments of the intestinal tract from esophagus to colon. Morphologically, the positive cells can be identified as fibroblasts and histiocytes/macrophages.

[0253] The same two cell types appear to display hybridization signal in connective tissue present in sections of other organs: skin, salivary glands, heart, prostate, portal tracts of liver.

[0254] A prominent hybridization signal was observed in the red pulp of spleen. The signal localized mainly to macrophages and to some lymphocytes. A similar pattern of expression (lymphocytes and macrophages) was also found in sinuses within the hillary region of the large lymph node. Another element of the lymphatic system, the thymus, also contains positive lymphocytes. Most of the positive lymphocytes concentrated in the medulla while the cortex scattered contained single positive cells. Scattered cells showing strong hybridization signal could be found in lung sections. Morphology of positive cells suggested that lung expression of the Ax1 gene is confined to the subset of macrophages and lung epithelial (type 1) cells. Expressing cells of both types can be found in the alveolar wall and within the alveolar and bronchial lumena. This pattern of lung cell expression suggested that activation of Ax1 expression preceded the "shedding" of these cell types.

[0255] In addition to the aforementioned portal tract cells, subsets of liver sinusoidal cells (endothelial, stellate and Kuppfer cells) also showed Ax1 expression. A weak hybridization signal was found in testis. This signal localized to some Sertoli cells and some germ cells within the basal layer of spermatogenic epithelium.

[0256] The Ax1-specific probe hybridized also to the sagittal section of the normal rat brain. Results of this hybridization suggested a rather low level of expression in the rat central nervous system. The only prominent site of "concentrated" expression was found in the cerebellum. A weak hybridization signal here localized to the "layer" of cells located at the border between molecular and granular layers. Comparisons with parallel sections stained with anti-MAP2 (neuronal marker) and anti-GFAP (astroglial marker) suggested glial specificity of Ax1 expression in this area. A weak hybridization signal was detected in single endothelial and probably glial cells scattered throughout other areas in the brain tissue.

[0257] Thus, in situ hybridization studies suggested rather widespread expression of Ax1 in rat tissues. The sites of expression were the interstitial and connective tissues present in many organs. The level of constitutive expression per cell appeared to be lower than that found in tubulointerstitial components of the fibrotic renal tissue after UUO or in chronic models.

EXAMPLE 7

Validation of Ax1 Activity and Relevance to Fibrosis in Cells

[0258] To examine the function of Ax1 in vitro several approaches are used:

[0259] 1. Overexpression of EGFR-Ax1 chimera in cells that are deficient in EGFR (NIH3T3-clone 2.2). Overexpressors were stimulated with EGF. Cellular response relevant for fibrosis is checked (e.g., collagen synthesis, fibroncetin expression).

[0260] 2. An expression vector harboring the Ax1 full open reading frame (Pires-Ax1) was used to obtain overexpressors of Ax1 in NIH3T3 cells. These cells are further used to analyze the effect of over-expression of Ax1 on cellular fibrosis response. Cellular response is checked (e.g., collagen synthesis, fibronectin expression).

[0261] 3. Ax1 is also transfected to NRK-49F and NRK-52E cells. The over-expressing cells obtained are used for the collagen assay and integrin expression is measured by FACS. These assays are performed following either TGF-β or GAS-6 stimulation (in NRK-F and NRKE, respectively).

EXAMPLE 8

In vivo Models for "Proof of Concept"

[0262] To establish the in vivo functional role of Ax1 in kidney fibrosis and glomerulosclerosis, mice in which the Ax1 gene was disrupted are used. These mice were generated by Profesor Goff in Columbia University and are obtained for the functional validation of Ax1. These mice are being used in order to evaluate kidney function following different models of kidney fibrosis and glomerulosclerosis (e.g., UUO), as compared to their normal counterparts exposed to the same treatment. Subsequently, kidney morphology, smooth muscle actin expression and collagen expression are being evaluated as measures of kidney function.

EXAMPLE 9

Immunostaining of Rat Kidney Samples With Anti-AXL Antibodies

[0263] Sections of UUO multiblock (including sham operated control, 24 hr, 5 d 10 d, 20 d and 25 d of UUO) and chronic renal failure (2 years 7 months old rat) were immunostained with anti AXL antibodies according to our established protocol (see methods section).

[0264] No immunostaining was observed in control (sham operated) sample. UUO samples demonstrated positive immunostaining at 24 hr-25 d. Most prominent staining was observed in apical part of tubular epithelial cells. Starting from 5 d of UUO immunostaining was observed also in interstitial cells. Sample from chronic renal failure kidney also demonstrated prominent tubulointerstitial immunostaining.

EXAMPLE 10

Screening Assays

[0265] A. Primary Cell Free in vitro Assay

[0266] Cell Free Assay Based on the Kinase Domain (hCytoAx1) of Ax1 Protein

[0267] A fluorescence polarization (FP)-based assay was developed for HTS of chemical libraries to identify small molecule inhibitors of Ax1 tyrosine kinase activity. The assay is based on detecting changes in fluorescence polar-

ization (FP) that occur as a result of substrate tyrosine phosphorylation. In this assay, the sustrate phosphorylated phosphorylated by Ax1 (competitor) competes for the binding of a fluorescein-labeled phosphopeptide (tracer) to a phosphotyrosine-specific antibody (pY-Ab). The unbound tracer displays low polarization, while its complex with the phosphotyrosine-specific antibody displays high polarization values due to restricted fluorophore rotation. Addition of a competitor to the tracer-Ab complex therefore results in fluorescence polarization decrease, which can be detected. Among the advantages of the fluorescence polarization technique for HTS are the relative insensitivity to changes in fluorescence intensity due to auto-fluorescence of chemical library components or their quenching effects. Additionally, FP is a homogenous technique that requires no separation of assay components prior to measurement.

[0268] Recombinant hCytoAx1 (cytoplasmic domain of the receptor tyrosine kinase, aa 495-894) was produced in insect cells (SF9 cells). For purification NiNTA matrix was used. 3 different substrates were tested as potential ax1 substrates in the assay:

[0269] Peptide 1—Biot-PDEILYVNMDE (major Ax1 autophosphorylation site)

[0270] Peptide 2—Biot-LSKKIYNGDYYR (Ax1 activation loop peptide)

[0271] PGT—Poly(Glu:Tyr) (4:1)—a universal, commonly-used tyrosine kinase substrate

[0272] hCytoAx1 from insect cells was immobilzed on beads. The beads bound protein was subjected to an in-vitro fluorescence polarization-based tyrosine kinase assay using poly(Glu:Tyr) as substrate and measurement of fluorescence polarization was performed. Activity of the immobilized protein was determined. Use of peptide 1 and peptide 2 is under investigation. Activity of a soluble purified protein is also being examined.

[0273] As an alternative to production of Ax1 protein from insect cells, Ax1 is also produced from bacteria expressing the protein. The recombinant hCyto Ax1 (cytoplasmic domain of the receptor tyrosine kinase, aa 495-894) was cloned.

[0274] To this end, 3 constructs were made:

[0275] GST—cytoAx1

[0276] GST—cyto Ax1 -His

[0277] GST cyto Ax1 K567R ("kinase dead")—as control.

[0278] All constructs showed high expression in bacteria. Glutathione affinity resin, or Ni NTA affinity resin followed by Glutathione affinity resin are used for purification to ensure specificity of hCytoAx1 preparation (devoid of other kinases). The purified protein from bacteria is used for the in vitro assay utilizing the same substrates and protocol described above for the insect cells derived Ax1 protein.

[0279] Cell Free Assay Based Full Length hAx1 Protein

[0280] DELFIA method (Wallac/PerkinElmer) based on dissociation of enhanced time-resolved fluorometric assay and enabling high sensitivity with wide dynamic range is employed for screening of hAx1 inhibitors in cell free assay. It is based on the tyrosine phosphorylation of substrate peptide by hAx1.

[0281] The method was established. The peptide used was—biotin-KKIYNGDYYRQGR (derived from Ax1 activation loop). hAx1-c-Myc protein (full length hAx1 with c-term myc tag) was expressed in 293 cells. Following cell lysis hAx1-c-Myc was immunoprecipitated with the 9B11 (anti-c-Myc tag antibody) and protein G-Sepharose. Immunocomplexs were used for in vitro kinase reaction in kinase assay buffer, 200 uM ATP and with 0.5 uM biotinylated peptide. Kinase reaction (1 hr) was stopped by the addition of EDTA and Delphia assay was preformed. Our results demonstrate high activity of immobilized hAx1-c-Myc towards its substrate in this assay. Activity of solubule protein from 293 cells is under analysis

[0282] B. Secondary Cell Based Assay

[0283] To evaluate the activity of Ax1 in the presense of inhibitors in a cell system, several approaches were taken.

[0284] The first was based on EGFR-hAx1 chimera (extracellular domain of Ax1 replaced by EGFR extracellular domain), using transient approach and STAT 3 for the reporter assay (STAT3 is a downstream target of Ax1). The readout of the assay was luminescence (Dual Luciferase Stop & Glo kit—Promega).

[0285] Cell lines used were NIH/3T3 (2.2) (devoid of endogenous EGFR expression) and 293T.

[0286] These were co-transfected with: EGFR -hAx1 chimera, STAT 3—Firefly Luciferase reporter (pSTAT 3-TA luc -Stratagene) as reporter of induction of Ax1 activity (TA-Luc vector served as control) and Renilla Luciferase (PRL-TK—Promega) to ensure specificity of signal generated by STAT 3. Cells transfected with EGFR-hAx1 kinase-dead (KD) mutant chimera, pSTAT 3-TA luc—Stratagene and pRL-TK—Promega served as specificity control.

[0287] 24 h post transfection the medium was replaced with "starvation" medium (DMEM with 0.5% BSA) for additional 24 hrs. Serum starvation protocol was employed in order to minimize the possibility that presence of EGF in the serum may cause EGFR-hAx1 chimera chimera aggregation, leading to its activation. Cells were activated with EGF (100 ng/mi) for 3 hrs (in serum-free medium) and then lysed. A sample from the cell lysates incubated with Firefly luciferase substrate followed by Renilla luciferase substrate (Stop & Glo dual Luciferase assay/Promega).

[0288] The results showed that transiently transfected EGFR-hAx1 chimera displayed autophosphorylation while the EGFR-hAx1 chimera kinase-dead (KD) mutant chimera transfected cells did not suggesting that Ax1 is active in the context of EGFR-hAx1 chimera. Ax1 inducible activation by EGF is being optimized by using different serum starvation protocols and optimization of transfection parameters. Following optimization the transient transfection protocol of EGFR-hAx1 chimera with the STAT3-luciferase reproter system is used for cell based assay following stimulation of ax1 activity by EGF.

[0289] An alternative approach for cell based assay relies also on STAT3 reporter-based assay but unlike the first approach-stably-transfected EGFR-hAx1 chimera cell clones showing autophosphorylation and EGF-inducible response are used. Both 293 and NIH3T3 cells are used to

produce stable clones of EGFR-hAx1 chimera and STAT 3—Firefly Luciferase reporter system is used as for the transient approach. 293 and NIH3T3 stably transfected with EGFR-hAx1 kinase-dead (KD) mutant chimera are used as control for specificity. These are also evaluated for ax1 activity in STAT3 reporter based assay as described above

[0290] A third alternative approach to EGFR-hAx1 chimera based bioassay (using EGF for stimulation), bioassay using the full length hAXL stimulated with GAS6 is evaluated.

[0291] NIH/3T3 (2.2) were transfected and stable clones expressing hAXL and hAXL inactive kinase mutant were generated. These showed no constitutive Ax1 tyrosine phosphorylation. In the assay the stable clone is transiently transfected with the STAT 3—fire fly Luciferase reporter (pSTAT 3-TA luc -Stratagene) and Renilla Luciferase (pRL-TK—Promega). Following transfection, GAS6 is used for stimulation of Ax1 activity which is measured by luminescence (Dual Luciferase Stop & Glo kit—Promega).

```
                          SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 6

<210> SEQ ID NO 1
<211> LENGTH: 5015
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 1

gagtggagtt ctggaggaat gtttaccaga cacagagccc agagggacag cgcccagagc      60

ccagatagag agacacggcc tcactggctc agcaccaggg tccccttccc cctcctcagc     120

tccctccctg gcccctttaa gaaagagctg atcctctcct ctcttgagtt aacccctgat     180

tgtccaggtg gcccctggct ctggcctggt gggcggaggc aaagggggag ccagggggcgg    240

agaaagggtt gcccaagtct gggagtgagg gaaggaggca ggggtgctga gaaggcggct     300

gctgggcaga gccggtggca agggcctccc ctgccgctgt gccaggcagg cagtgccaaa     360

tccggggagc ctggagctgg ggggagggcc ggggacagcc cggcccgctg ccccctcccc     420

cgctgggagc ccagcaactt ctgaggaaag tttggcaccc atggcgtggc ggtgccccag     480

gatgggcagg gtcccgctgg cctggtgctt ggcgctgtgc ggctgggcgt gcatggcccc     540

caggggcacg caggctgaag aaagtccctt cgtgggcaac ccagggaata tcacaggtgc     600

ccggggactc acgggcaccc ttcggtgtca gctccaggtt cagggagagc cccccgaggt     660

acattggctt cgggatggac agatcctgga gctcgcggac agcacccaga cccaggtgcc     720

cctgggtgag gatgaacagg atgactggat agtggtcagc cagctcagaa tcacctccct     780

gcagctttcc gacacgggac agtaccagtg tttggtgttt ctgggacatc agaccttcgt     840

gtcccagcct ggctatgttg ggctggaggg cttgccttac ttcctggagg agcccgaaga     900

caggactgtg gccgccaaca ccccctcaa cctgagctgc caagctcagg gacccccaga     960

gcccgtggac ctactctggc tccaggatgc tgtcccctg gccacggctc caggtcacgg    1020

cccccagcgc agcctgcatg ttccagggct gaacaagaca tcctctttct cctgcgaagc    1080

ccataacgcc aaggggggtca ccacatcccg cacagccacc atcacagtgc tcccccagca    1140

gccccgtaac ctccacctgg tctcccgcca acccacggag ctggaggtgg cttggactcc    1200

aggcctgagc ggcatctacc ccctgaccca ctgcaccctg caggctgtgc tgtcagacga    1260

tgggatgggc atccaggcgg gagaaccaga ccccccagag gagcccctca cctcgcaagc    1320

atccgtgccc ccccatcagc ttcggctagg cagcctccat cctcaccccc cttatcacat    1380

ccgcgtggca tgcaccagca gccagggccc ctcatcctgg acccactggc ttcctgtgga    1440

gacgccggag ggagtgcccc tgggcccccc tgagaacatt agtgctacgc ggaatgggag    1500
```

-continued

```
ccaggccttc gtgcattggc aagagccccg ggcgcccctg cagggtaccc tgttagggta   1560
ccggctggcg tatcaaggcc aggacacccc agaggtgcta atggacatag ggctaaggca   1620
agaggtgacc ctggagctgc agggggacgg gtctgtgtcc aatctgacag tgtgtgtggc   1680
agcctacact gctgctgggg atggaccctg gagcctccca gtacccctgg aggcctggcg   1740
cccaggggaa gcacagccag tccaccagct ggtgaaggaa ccttcaactc ctgccttctc   1800
gtggccctgg tggtatgtac tgctaggagc agtcgtggcc gctgcctgtg tcctcatctt   1860
ggctctcttc cttgtccacc ggcgaaagaa ggagacccgt tatggagaag tgtttgaacc   1920
aacagtggaa agaggtgaac tggtagtcag gtaccgcgtg cgcaagtcct acagtcgtcg   1980
gaccactgaa gctaccttga acagcctggg catcagtgaa gagctgaagg agaagctgcg   2040
ggatgtgatg gtggaccggc acaaggtggc cctggggaag actctgggag agggagagtt   2100
tggagctgtg atggaaggcc agctcaacca ggacgactcc atcctcaagg tggctgtgaa   2160
gacgatgaag attgccatct gcacgaggtc agagctggag gatttcctga gtgaagcggt   2220
ctgcatgaag gaatttgacc atcccaacgt catgaggctc atcggtgtct gtttccaggg   2280
ttctgaacga gagagcttcc cagcacctgt ggtcatctta cctttcatga aacatggaga   2340
cctacacagc ttcctcctct attcccggct cgggggccag ccagtgtacc tgcccactca   2400
gatgctagtg aagttcatgg cagacatcgc cagtggcatg gagtatctga gtaccaagag   2460
attcatacac cgggacctgg cggccaggaa ctgcatgctg aatgagaaca tgtccgtgtg   2520
tgtggcggac ttcgggctct ccaagaagat ctacaatggg gactactacc gccagggacg   2580
tatcgccaag atgccagtca agtggattgc cattgagagt ctagctgacc gtgtctacac   2640
cagcaagagc gatgtgtggt ccttcggggt gacaatgtgg gagattgcca caagaggcca   2700
aaccccatat ccgggcgtgg agaacagcga gatttatgac tatctgcgcc agggaaatcg   2760
cctgaagcag cctgcggact gtctggatgg actgtatgcc ttgatgtcgc ggtgctggga   2820
gctaaatccc caggaccggc caagttttac agagctgcgg gaagatttgg agaacacact   2880
gaaggccttg cctcctgccc aggagcctga cgaaatcctc tatgtcaaca tggatgaggg   2940
tggaggttat cctgaacccc ctggagctgc aggaggagct gaccccccaa cccagccaga   3000
ccctaaggat tcctgtagct gcctcactgc ggctgaggtc catcctgctg gacgctatgt   3060
cctctgccct tccacaaccc ctagccccgc tcagcctgct gataggggct ccccagcagc   3120
cccagggcag gaggatggtg cctgagacaa ccctccacct ggtactccct ctcaggatcc   3180
aagctaagca ctgccactgg gggaaactcc accttcccac tttcccaccc cacgccttat   3240
ccccacttgc agccctgtct tcctacctat cccacctcca tcccagacag gtccctggcc   3300
ttctctgtgc agtagcatca ccttgaaagc agtagcatca ccatctgtaa aaggaagggg   3360
ttggattgca atatctgaag ccctcccagg tgttaacatt ccaagactct agagtccaag   3420
gtttaaagag tctagattca aaggttctag gtttcaaaga tgctgtgagt ctttggttct   3480
aaggacctga aattccaaag tctctaattc tattaaagtg ctaaggttct aaggcctact   3540
tttttttttt tttttttttt tttttttttt tttgcgatag agtctcactg tgtcacccag   3600
gctggagtgc agtggtgcaa tctcgcctca ctgcaacctt cacctaccga gttcaagtga   3660
ttttcctgcc ttggcctccc aagtagctgg gattacaggt gtgtgccacc acacccggct   3720
aatttttata tttttagtag agacagggtt tcaccatgtt ggccaggctg gtctaaaact   3780
```

-continued

```
cctgacctca agtgatctgc ccacctcagc ctcccaaagt gctgagatta caggcatgag   3840

ccactgcact caaccttaag acctactgtt ctaaagctct gacattatgt ggttttagat   3900

tttctggttc taacattttt gataaagcct caaggtttta ggttctaaag ttctaagatt   3960

ctgattttag gagctaaggc tctatgagtc tagatgttta ttcttctaga gttcagagtc   4020

cttaaaatgt aagattatag attctaaaga ttctatagtt ctagacatgg aggttctaag   4080

gcctaggatt ctaaaatgtg atgttctaag gctctgagag tctagattct ctggctgtaa   4140

ggctctagat cataaggctt caaaatgtta tcttctcaag ttctaagatt ctaatgatga   4200

tcaattatag tttctgaggc tttatgataa tagattctct tgtataagat cctagatcct   4260

aagggtcgaa agctctagaa tctgcaattc aaaagttcca agagtctaaa gatggagttt   4320

ctaaggtccg gtgttctaag atgtgatatt ctaagactta ctctaagatc ttagattctc   4380

tgtgtctaag attctagatc agatgctcca agattctaga tgattaaata agattctaac   4440

ggtctgttct gtttcaaggc actctagatt ccattggtcc aagattccgg atcctaagca   4500

tctaagttat aagactctca cactcagttg tgactaacta gacaccaaag ttctaataat   4560

ttctaatgtt ggacaccttt aggttctttg ctssattctg cctctctagg accatggtta   4620

agagtccaag aatccacatt tctaaaatct tatagttcta ggcactgtag ttctaagact   4680

caaatgttct aagtttctaa gattctaaag gtccacaggt ctagactatt aggtgcaatt   4740

tcaaggttct aaccctatac tgtagtattc tttggggtgc cctctccctt cttagctatc   4800

attgcttcct cctccccaac tgtgggggtg tgccccctte aagcctgtgc aatgcattag   4860

ggatgcctcc tttccgcagg ggatggacga tctcccacct ttcgggccat gttgcccccg   4920

tgagccaatc cctcaccttc tgagtacaga gtgtggactc tggtgcctcc agaggggctc   4980

aggtcacata aaactttgta tatcaacgaa aaaaa                              5015
```

<210> SEQ ID NO 2
<211> LENGTH: 894
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 2

```
Met Ala Trp Arg Cys Pro Arg Met Gly Arg Val Pro Leu Ala Trp Cys
1               5                   10                  15

Leu Ala Leu Cys Gly Trp Ala Cys Met Ala Pro Arg Gly Thr Gln Ala
            20                  25                  30

Glu Glu Ser Pro Phe Val Gly Asn Pro Gly Asn Ile Thr Gly Ala Arg
        35                  40                  45

Gly Leu Thr Gly Thr Leu Arg Cys Gln Leu Gln Val Gln Gly Glu Pro
    50                  55                  60

Pro Glu Val His Trp Leu Arg Asp Gly Gln Ile Leu Glu Leu Ala Asp
65                  70                  75                  80

Ser Thr Gln Thr Gln Val Pro Leu Gly Glu Asp Glu Gln Asp Asp Trp
                85                  90                  95

Ile Val Val Ser Gln Leu Arg Ile Thr Ser Leu Gln Leu Ser Asp Thr
            100                 105                 110

Gly Gln Tyr Gln Cys Leu Val Phe Leu Gly His Gln Thr Phe Val Ser
        115                 120                 125

Gln Pro Gly Tyr Val Gly Leu Glu Gly Leu Pro Tyr Phe Leu Glu Glu
    130                 135                 140
```

-continued

```
Pro Glu Asp Arg Thr Val Ala Ala Asn Thr Pro Phe Asn Leu Ser Cys
145                 150                 155                 160

Gln Ala Gln Gly Pro Pro Glu Pro Val Asp Leu Leu Trp Leu Gln Asp
                165                 170                 175

Ala Val Pro Leu Ala Thr Ala Pro Gly His Gly Pro Gln Arg Ser Leu
            180                 185                 190

His Val Pro Gly Leu Asn Lys Thr Ser Ser Phe Ser Cys Glu Ala His
        195                 200                 205

Asn Ala Lys Gly Val Thr Thr Ser Arg Thr Ala Thr Ile Thr Val Leu
    210                 215                 220

Pro Gln Gln Pro Arg Asn Leu His Leu Val Ser Arg Gln Pro Thr Glu
225                 230                 235                 240

Leu Glu Val Ala Trp Thr Pro Gly Leu Ser Gly Ile Tyr Pro Leu Thr
                245                 250                 255

His Cys Thr Leu Gln Ala Val Leu Ser Asp Asp Gly Met Gly Ile Gln
            260                 265                 270

Ala Gly Glu Pro Asp Pro Pro Glu Glu Pro Leu Thr Ser Gln Ala Ser
        275                 280                 285

Val Pro Pro His Gln Leu Arg Leu Gly Ser Leu His Pro His Pro Pro
    290                 295                 300

Tyr His Ile Arg Val Ala Cys Thr Ser Ser Gln Gly Pro Ser Ser Trp
305                 310                 315                 320

Thr His Trp Leu Pro Val Glu Thr Pro Glu Gly Val Pro Leu Gly Pro
                325                 330                 335

Pro Glu Asn Ile Ser Ala Thr Arg Asn Gly Ser Gln Ala Phe Val His
            340                 345                 350

Trp Gln Glu Pro Arg Ala Pro Leu Gln Gly Thr Leu Leu Gly Tyr Arg
        355                 360                 365

Leu Ala Tyr Gln Gly Gln Asp Thr Pro Glu Val Leu Met Asp Ile Gly
    370                 375                 380

Leu Arg Gln Glu Val Thr Leu Glu Leu Gln Gly Asp Gly Ser Val Ser
385                 390                 395                 400

Asn Leu Thr Val Cys Val Ala Ala Tyr Thr Ala Ala Gly Asp Gly Pro
                405                 410                 415

Trp Ser Leu Pro Val Pro Leu Glu Ala Trp Arg Pro Gly Glu Ala Gln
            420                 425                 430

Pro Val His Gln Leu Val Lys Glu Pro Ser Thr Pro Ala Phe Ser Trp
        435                 440                 445

Pro Trp Trp Tyr Val Leu Leu Gly Ala Val Val Ala Ala Ala Cys Val
    450                 455                 460

Leu Ile Leu Ala Leu Phe Leu Val His Arg Arg Lys Lys Glu Thr Arg
465                 470                 475                 480

Tyr Gly Glu Val Phe Glu Pro Thr Val Glu Arg Gly Glu Leu Val Val
                485                 490                 495

Arg Tyr Arg Val Arg Lys Ser Tyr Ser Arg Arg Thr Thr Glu Ala Thr
            500                 505                 510

Leu Asn Ser Leu Gly Ile Ser Glu Glu Leu Lys Glu Lys Leu Arg Asp
        515                 520                 525

Val Met Val Asp Arg His Lys Val Ala Leu Gly Lys Thr Leu Gly Glu
    530                 535                 540
```

-continued

```
Gly Glu Phe Gly Ala Val Met Glu Gly Gln Leu Asn Gln Asp Asp Ser
545                 550                 555                 560

Ile Leu Lys Val Ala Val Lys Thr Met Lys Ile Ala Ile Cys Thr Arg
                565                 570                 575

Ser Glu Leu Glu Asp Phe Leu Ser Glu Ala Val Cys Met Lys Glu Phe
            580                 585                 590

Asp His Pro Asn Val Met Arg Leu Ile Gly Val Cys Phe Gln Gly Ser
        595                 600                 605

Glu Arg Glu Ser Phe Pro Ala Pro Val Val Ile Leu Pro Phe Met Lys
    610                 615                 620

His Gly Asp Leu His Ser Phe Leu Leu Tyr Ser Arg Leu Gly Gly Gln
625                 630                 635                 640

Pro Val Tyr Leu Pro Thr Gln Met Leu Val Lys Phe Met Ala Asp Ile
                645                 650                 655

Ala Ser Gly Met Glu Tyr Leu Ser Thr Lys Arg Phe Ile His Arg Asp
            660                 665                 670

Leu Ala Ala Arg Asn Cys Met Leu Asn Glu Asn Met Ser Val Cys Val
        675                 680                 685

Ala Asp Phe Gly Leu Ser Lys Lys Ile Tyr Asn Gly Asp Tyr Tyr Arg
    690                 695                 700

Gln Gly Arg Ile Ala Lys Met Pro Val Lys Trp Ile Ala Ile Glu Ser
705                 710                 715                 720

Leu Ala Asp Arg Val Tyr Thr Ser Lys Ser Asp Val Trp Ser Phe Gly
                725                 730                 735

Val Thr Met Trp Glu Ile Ala Thr Arg Gly Gln Thr Pro Tyr Pro Gly
            740                 745                 750

Val Glu Asn Ser Glu Ile Tyr Asp Tyr Leu Arg Gln Gly Asn Arg Leu
        755                 760                 765

Lys Gln Pro Ala Asp Cys Leu Asp Gly Leu Tyr Ala Leu Met Ser Arg
    770                 775                 780

Cys Trp Glu Leu Asn Pro Gln Asp Arg Pro Ser Phe Thr Glu Leu Arg
785                 790                 795                 800

Glu Asp Leu Glu Asn Thr Leu Lys Ala Leu Pro Pro Ala Gln Glu Pro
                805                 810                 815

Asp Glu Ile Leu Tyr Val Asn Met Asp Glu Gly Gly Gly Tyr Pro Glu
            820                 825                 830

Pro Pro Gly Ala Ala Gly Gly Ala Asp Pro Pro Thr Gln Pro Asp Pro
        835                 840                 845

Lys Asp Ser Cys Ser Cys Leu Thr Ala Ala Glu Val His Pro Ala Gly
    850                 855                 860

Arg Tyr Val Leu Cys Pro Ser Thr Thr Pro Ser Pro Ala Gln Pro Ala
865                 870                 875                 880

Asp Arg Gly Ser Pro Ala Ala Pro Gly Gln Glu Asp Gly Ala
                885                 890
```

<210> SEQ ID NO 3
<211> LENGTH: 4986
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 3

```
gagtggagtt ctggaggaat gtttaccaga cacagagccc agagggacag cgcccagagc     60
```

-continued

```
ccagatagag agacacggcc tcactggctc agcaccaggg tccccttccc cctcctcagc     120
tccctccctg gcccctttaa gaaagagctg atcctctcct ctcttgagtt aacccctgat     180
tgtccaggtg gcccctggct ctggcctggt gggcggaggc aaagggggag ccaggggcgg     240
agaaagggtt gcccaagtct gggagtgagg gaaggaggca ggggtgctga gaaggcggct     300
gctgggcaaa gccggtggca agggcctccc ctgccgctgt gccaggcagg cagtgccaaa     360
tccggggagc ctggagctgg ggggagggcc ggggacagcc cggccctgcc ccctcccccg     420
ctgggagccc agcaacttct gaggaaagtt tggcacccat ggcgtggcgg tgccccagga     480
tgggcagggt cccgctggcc tggtgcttgg cgctgtgcgg ctgggcgtgc atggccccca     540
ggggcacgca ggctgaagaa agtcccttcg tgggcaaccc agggaatatc acaggtgccc     600
ggggactcac gggcaccctt cggtgtcagc tccaggttca gggagagccc cccgaggtac     660
attggcttcg ggatggacag atcctggagc tcgcggacag cacccagacc caggtgcccc     720
tgggtgagga tgaacaggat gactggatag tggtcagcca gctcagaatc acctccctgc     780
agctttccga cacgggacag taccagtgtt tggtgtttct gggacatcag accttcgtgt     840
cccagcctgg ctatgttggg ctggagggct tgccttactt cctggaggag cccgaagaca     900
ggactgtggc cgccaacacc cccttcaacc tgagctgcca agctcaggga cccccagagc     960
ccgtggacct actctggctc caggatgctg tcccccctggc cacggctcca ggtcacggcc    1020
cccagcgcag cctgcatgtt ccagggctga acaagacatc ctctttctcc tgcgaagccc    1080
ataacgccaa gggggtcacc acatcccgca cagccaccat cacagtgctc ccccagcagc    1140
cccgtaacct ccacctggtc tcccgccaac ccacggagct ggaggtggct tggactccag    1200
gcctgagcgg catctacccc ctgacccact gcaccctgca ggctgtgctg tcagacgatg    1260
ggatgggcat ccaggcggga gaaccagacc ccccagagga gcccctcacc tcgcaagcat    1320
ccgtgccccc ccatcagctt cggctaggca gcctccatcc tcacacccct tatcacatcc    1380
gcgtggcatg caccagcagc cagggcccct catcctggac ccactggctt cctgtggaga    1440
cgccggaggg agtgcccctg ggccccccta agaacattag tgctacgcgg aatgggagcc    1500
aggccttcgt gcattggcaa gagccccggg cgcccctgca gggtaccctg ttagggtacc    1560
ggctggcgta tcaaggccag gacaccccag aggtgctaat ggacataggg ctaaggcaag    1620
aggtgaccct ggagctgcag ggggacgggt ctgtgtccaa tctgacagtg tgtgtggcag    1680
cctacactgc tgctggggat ggaccctgga gcctcccagt accccctggag gcctggcgcc    1740
cagtgaagga accttcaact cctgccttct cgtggccctg gtggtatgta ctgctaggag    1800
cagtcgtggc cgctgcctgt gtcctcatct tggctctctt ccttgtccac cggcgaaaga    1860
aggagacccg ttatggagaa gtgtttgaac caacagtgga aagaggtgaa ctggtagtca    1920
ggtaccgcgt gcgcaagtcc tacagtcgtc ggaccactga agctaccttg aacagcctgg    1980
gcatcagtga agagctgaag gagaagctgc gggatgtgat ggtggaccgg cacaaggtgg    2040
ccctggggaa gactctggga gagggagagt ttggagctgt gatggaaggc cagctcaacc    2100
aggacgactc catcctcaag gtggctgtga agacgatgaa gattgccatc tgcacgaggt    2160
cagagctgga ggatttcctg agtgaagcgg tctgcatgaa ggaatttgac catcccaacg    2220
tcatgaggct catcggtgtc tgtttccagg gttctgaacg agagagcttc ccagcacctg    2280
tggtcatctt acctttcatg aaacatggag acctacacag cttcctcctc tattcccggc    2340
```

-continued

```
tcggggacca gccagtgtac ctgcccactc agatgctagt gaagttcatg gcagacatcg   2400
ccagtggcat ggagtatctg agtaccaaga gattcataca ccgggacctg gcggccagga   2460
actgcatgct gaatgagaac atgtccgtgt gtgtggcgga cttcgggctc tccaagaaga   2520
tctacaatgg ggactactac cgccagggac gtatcgccaa gatgccagtc aagtggattg   2580
ccattgagag tctagctgac cgtgtctaca ccagcaagag cgatgtgtgg tccttcgggg   2640
tgacaatgtg ggagattgcc acaagaggcc aaaccccata tccgggcgtg gagaacagcg   2700
agatttatga ctatctgcgc cagggaaatc gcctgaagca gcctgcggac tgtctggatg   2760
gactgtatgc cttgatgtcg cggtgctggg agctaaatcc ccaggaccgg ccaagtttta   2820
cagagctgcg ggaagatttg gagaacacac tgaaggcctt gcctcctgcc caggagcctg   2880
acgaaatcct ctatgtcaac atggatgagg gtggaggtta tcctgaaccc cctggagctg   2940
caggaggagc tgacccccca acccagccag accctaagga ttcctgtagc tgcctcactg   3000
cggctgaggt ccatcctgct ggacgctatg tcctctgccc ttccacaacc cctagccccg   3060
ctcagcctgc tgataggggc tccccagcag ccccagggca ggaggatggt gcctgagaca   3120
accctccacc tggtactccc tctcaggatc caagctaagc actgccactg gggaaaactc   3180
caccttccca cttttccacc ccacgcctta tccccacttg cagccctgtc ttcctaccta   3240
tcccacctcc atcccagaca ggtccctccc cttctctgtg cagtagcatc accttgaaag   3300
cagtagcatc accatctgta aaaggaaggg gttggattgc aatatctgaa gccctcccag   3360
gtgttaacat tccaagactc tagagtccaa ggtttaaaga gtctagattc aaaggttcta   3420
ggtttcaaag atgctgtgag tctttggttc taaggacctg aaattccaaa gtctctaatt   3480
ctattaaagt gctaaggttc taaggcctac tttttttttt tttttttttt tttttttttt   3540
ttttgcgata gagtctcact gtgtcaccca ggctggagtg cagtggtgca atctcgcctc   3600
actgcaacct tcacctaccg agttcaagtg attttcctgc cttggcctcc caagtagctg   3660
ggattacagg tgtgtgccac cacacccggc taattttat attttagta gagacagggt     3720
ttcaccatgt tggccaggct ggtctaaaac tcctgacctc aagtgatctg cccacctcag   3780
cctcccaaag tgctgagatt acaggcatga gccactgcac tcaaccttaa gacctactgt   3840
tctaaagctc tgacattatg tggttttaga ttttctggtt ctaacatttt tgataaagcc   3900
tcaaggtttt aggttctaaa gttctaagat tctgatttta ggagctaagg ctctatgagt   3960
ctagatgttt attcttctag agttcagagt ccttaaaatg taagattata gattctaaag   4020
attctatagt tctagacatg gaggttctaa ggcctaggat tctaaaatgt gatgttctaa   4080
ggctctgaga gtctagattc tctggctgta aggctctaga tcataaggct tcaaaatgtt   4140
atcttctcaa gttctaagat tctaatgatg atcaattata gtttctgagg ctttatgata   4200
atagattctc ttgtataaga tcctagatcc taagggtcga aagctctaga atctgcaatt   4260
caaaagttcc aagagtctaa agatggagtt tctaaggtcc ggtgttctaa gatgtgatat   4320
tctaagactt actctaagat cttagattct ctgtgtctaa gattctagat cagatgctcc   4380
aagattctag atgattaaat aagattctaa cggtctgttc tgtttcaagg cactctagat   4440
tccattggtc caagattccg gatcctaagc atctaagtta taagactctc acactcagtt   4500
gtgactaact agacaccaaa gttctaataa tttctaatgt tggacacctt taggttcttt   4560
gctssattct gcctctctag gaccatggtt aagagtccaa gaatccacat ttctaaaatc   4620
```

-continued

ttatagttct aggcactgta gttctaagac tcaaatgttc taagtttcta agattctaaa 4680

ggtccacagg tctagactat taggtgcaat ttcaaggttc taaccctata ctgtagtatt 4740

ctttggggtg cccctctcct tcttagctat cattgcttcc tcctccccaa ctgtgggggt 4800

gtgccccctt caagcctgtg caatgcatta gggatgcctc ctttccgcag gggatggacg 4860

atctcccacc tttcgggcca tgttgccccc gtgagccaat ccctcacctt ctgagtacag 4920

agtgtggact ctggtgcctc cagaggggct caggtcacat aaaactttgt atatcaacga 4980

aaaaaa 4986

<210> SEQ ID NO 4
<211> LENGTH: 885
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 4

Met Ala Trp Arg Cys Pro Arg Met Gly Arg Val Pro Leu Ala Trp Cys
1 5 10 15

Leu Ala Leu Cys Gly Trp Ala Cys Met Ala Pro Arg Gly Thr Gln Ala
20 25 30

Glu Glu Ser Pro Phe Val Gly Asn Pro Gly Asn Ile Thr Gly Ala Arg
35 40 45

Gly Leu Thr Gly Thr Leu Arg Cys Gln Leu Gln Val Gln Gly Glu Pro
50 55 60

Pro Glu Val His Trp Leu Arg Asp Gly Gln Ile Leu Glu Leu Ala Asp
65 70 75 80

Ser Thr Gln Thr Gln Val Pro Leu Gly Glu Asp Glu Gln Asp Asp Trp
85 90 95

Ile Val Val Ser Gln Leu Arg Ile Thr Ser Leu Gln Leu Ser Asp Thr
100 105 110

Gly Gln Tyr Gln Cys Leu Val Phe Leu Gly His Gln Thr Phe Val Ser
115 120 125

Gln Pro Gly Tyr Val Gly Leu Glu Gly Leu Pro Tyr Phe Leu Glu Glu
130 135 140

Pro Glu Asp Arg Thr Val Ala Ala Asn Thr Pro Phe Asn Leu Ser Cys
145 150 155 160

Gln Ala Gln Gly Pro Pro Glu Pro Val Asp Leu Leu Trp Leu Gln Asp
165 170 175

Ala Val Pro Leu Ala Thr Ala Pro Gly His Gly Pro Gln Arg Ser Leu
180 185 190

His Val Pro Gly Leu Asn Lys Thr Ser Ser Phe Ser Cys Glu Ala His
195 200 205

Asn Ala Lys Gly Val Thr Thr Ser Arg Thr Ala Thr Ile Thr Val Leu
210 215 220

Pro Gln Gln Pro Arg Asn Leu His Leu Val Ser Arg Gln Pro Thr Glu
225 230 235 240

Leu Glu Val Ala Trp Thr Pro Gly Leu Ser Gly Ile Tyr Pro Leu Thr
245 250 255

His Cys Thr Leu Gln Ala Val Leu Ser Asp Asp Gly Met Gly Ile Gln
260 265 270

Ala Gly Glu Pro Asp Pro Pro Glu Glu Pro Leu Thr Ser Gln Ala Ser
275 280 285

-continued

```
Val Pro Pro His Gln Leu Arg Leu Gly Ser Leu His Pro His Thr Pro
    290                 295                 300

Tyr His Ile Arg Val Ala Cys Thr Ser Ser Gln Gly Pro Ser Ser Trp
305                 310                 315                 320

Thr His Trp Leu Pro Val Glu Thr Pro Glu Gly Val Pro Leu Gly Pro
                325                 330                 335

Pro Lys Asn Ile Ser Ala Thr Arg Asn Gly Ser Gln Ala Phe Val His
            340                 345                 350

Trp Gln Glu Pro Arg Ala Pro Leu Gln Gly Thr Leu Leu Gly Tyr Arg
        355                 360                 365

Leu Ala Tyr Gln Gly Gln Asp Thr Pro Glu Val Leu Met Asp Ile Gly
    370                 375                 380

Leu Arg Gln Glu Val Thr Leu Glu Leu Gln Gly Asp Gly Ser Val Ser
385                 390                 395                 400

Asn Leu Thr Val Cys Val Ala Ala Tyr Thr Ala Ala Gly Asp Gly Pro
                405                 410                 415

Trp Ser Leu Pro Val Pro Leu Glu Ala Trp Arg Pro Val Lys Glu Pro
            420                 425                 430

Ser Thr Pro Ala Phe Ser Trp Pro Trp Trp Tyr Val Leu Leu Gly Ala
        435                 440                 445

Val Val Ala Ala Ala Cys Val Leu Ile Leu Ala Leu Phe Leu Val His
    450                 455                 460

Arg Arg Lys Lys Glu Thr Arg Tyr Gly Glu Val Phe Glu Pro Thr Val
465                 470                 475                 480

Glu Arg Gly Glu Leu Val Val Arg Tyr Arg Val Arg Lys Ser Tyr Ser
                485                 490                 495

Arg Arg Thr Thr Glu Ala Thr Leu Asn Ser Leu Gly Ile Ser Glu Glu
            500                 505                 510

Leu Lys Glu Lys Leu Arg Asp Val Met Val Asp Arg His Lys Val Ala
        515                 520                 525

Leu Gly Lys Thr Leu Gly Glu Gly Glu Phe Gly Ala Val Met Glu Gly
    530                 535                 540

Gln Leu Asn Gln Asp Asp Ser Ile Leu Lys Val Ala Val Lys Thr Met
545                 550                 555                 560

Lys Ile Ala Ile Cys Thr Arg Ser Glu Leu Glu Asp Phe Leu Ser Glu
                565                 570                 575

Ala Val Cys Met Lys Glu Phe Asp His Pro Asn Val Met Arg Leu Ile
            580                 585                 590

Gly Val Cys Phe Gln Gly Ser Glu Arg Glu Ser Phe Pro Ala Pro Val
        595                 600                 605

Val Ile Leu Pro Phe Met Lys His Gly Asp Leu His Ser Phe Leu Leu
    610                 615                 620

Tyr Ser Arg Leu Gly Asp Gln Pro Val Tyr Leu Pro Thr Gln Met Leu
625                 630                 635                 640

Val Lys Phe Met Ala Asp Ile Ala Ser Gly Met Glu Tyr Leu Ser Thr
                645                 650                 655

Lys Arg Phe Ile His Arg Asp Leu Ala Ala Arg Asn Cys Met Leu Asn
            660                 665                 670

Glu Asn Met Ser Val Cys Val Ala Asp Phe Gly Leu Ser Lys Lys Ile
        675                 680                 685

Tyr Asn Gly Asp Tyr Tyr Arg Gln Gly Arg Ile Ala Lys Met Pro Val
```

-continued

```
    690                 695                 700

Lys Trp Ile Ala Ile Glu Ser Leu Ala Asp Arg Val Tyr Thr Ser Lys
705                 710                 715                 720

Ser Asp Val Trp Ser Phe Gly Val Thr Met Trp Glu Ile Ala Thr Arg
                725                 730                 735

Gly Gln Thr Pro Tyr Pro Gly Val Glu Asn Ser Glu Ile Tyr Asp Tyr
            740                 745                 750

Leu Arg Gln Gly Asn Arg Leu Lys Gln Pro Ala Asp Cys Leu Asp Gly
        755                 760                 765

Leu Tyr Ala Leu Met Ser Arg Cys Trp Glu Leu Asn Pro Gln Asp Arg
    770                 775                 780

Pro Ser Phe Thr Glu Leu Arg Glu Asp Leu Glu Asn Thr Leu Lys Ala
785                 790                 795                 800

Leu Pro Pro Ala Gln Glu Pro Asp Glu Ile Leu Tyr Val Asn Met Asp
                805                 810                 815

Glu Gly Gly Gly Tyr Pro Glu Pro Pro Gly Ala Ala Gly Gly Ala Asp
            820                 825                 830

Pro Pro Thr Gln Pro Asp Pro Lys Asp Ser Cys Ser Cys Leu Thr Ala
        835                 840                 845

Ala Glu Val His Pro Ala Gly Arg Tyr Val Leu Cys Pro Ser Thr Thr
    850                 855                 860

Pro Ser Pro Ala Gln Pro Ala Asp Arg Gly Ser Pro Ala Ala Pro Gly
865                 870                 875                 880

Gln Glu Asp Gly Ala
                885


<210> SEQ ID NO 5
<211> LENGTH: 268
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: PEPTIDE
<222> LOCATION: (1)..(268)

<400> SEQUENCE: 5

Val Ala Leu Gly Lys Thr Leu Gly Glu Gly Glu Phe Gly Ala Val Met
1               5                   10                  15

Glu Gly Gln Leu Asn Gln Asp Asp Ser Ile Leu Lys Val Ala Val Lys
            20                  25                  30

Thr Met Lys Ile Ala Ile Cys Thr Arg Ser Glu Leu Glu Asp Phe Leu
        35                  40                  45

Ser Glu Ala Val Cys Met Lys Glu Phe Asp His Pro Asn Val Met Arg
    50                  55                  60

Leu Ile Gly Val Cys Phe Gln Gly Ser Glu Arg Glu Ser Phe Pro Ala
65                  70                  75                  80

Pro Val Val Ile Leu Pro Phe Met Lys His Gly Asp Leu His Ser Phe
                85                  90                  95

Leu Leu Tyr Ser Arg Leu Gly Asp Gln Pro Val Tyr Leu Pro Thr Gln
            100                 105                 110

Met Leu Val Lys Phe Met Ala Asp Ile Ala Ser Gly Met Glu Tyr Leu
        115                 120                 125

Ser Thr Lys Arg Phe Ile His Arg Asp Leu Ala Ala Arg Asn Cys Met
    130                 135                 140
```

-continued

```
Leu Asn Glu Asn Met Ser Val Cys Val Ala Asp Phe Gly Leu Ser Lys
145                 150                 155                 160

Lys Ile Tyr Asn Gly Asp Tyr Tyr Arg Gln Gly Arg Ile Ala Lys Met
                165                 170                 175

Pro Val Lys Trp Ile Ala Ile Glu Ser Leu Ala Asp Arg Val Tyr Thr
            180                 185                 190

Ser Lys Ser Asp Val Trp Ser Phe Gly Val Thr Met Trp Glu Ile Ala
        195                 200                 205

Thr Arg Gly Gln Thr Pro Tyr Pro Gly Val Glu Asn Ser Glu Ile Tyr
    210                 215                 220

Asp Tyr Leu Arg Gln Gly Asn Arg Leu Lys Gln Pro Ala Asp Cys Leu
225                 230                 235                 240

Asp Gly Leu Tyr Ala Leu Met Ser Arg Cys Trp Glu Leu Asn Pro Gln
                245                 250                 255

Asp Arg Pro Ser Phe Thr Glu Leu Arg Glu Asp Leu
            260                 265


<210> SEQ ID NO 6
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: PEPTIDE
<222> LOCATION: (1)..(7)

<400> SEQUENCE: 6

Lys Trp Ile Ala Ile Glu Ser
1               5
```

What is claimed is:

1. A process of identifying a compound capable of inhibiting the activity of a human Ax1 receptor that comprises the steps of:

(iv) contacting the Ax1 receptor or cells expressing the Ax1 receptor with the compound;

(v) measuring the Ax1 receptor activity in the presence of the compound and

(vi) comparing the activity measured in step (ii) to that measured in the absence of the compound under controlled conditions, wherein a decrease identifies the compound as being capable of inhibiting the activity.

2. The process of claim 1, wherein the activity measured is tyrosine phosphorylation of a substrate of the Ax1 receptor.

3. The process of claim 1, wherein the activity measured is auto phosphorylation of the Ax1 receptor.

4. The process of claim 1, wherein the cells are contacted with the compound.

5. The process of claim 4, wherein the cells contacted with the compound are mesangial cells and the activity measured is proliferation of said mesangial cells.

6. The process of claim 4, wherein the cells contacted with the compound are renal fibroblasts and the activity measured is proliferation of said renal fibroblasts.

7. The process of claim 4, wherein the cells contacted with the compound are renal fibroblasts and the activity measured is collagen deposition in the extracellular matrix of said renal fibroblasts.

8. The process of claim 4, wherein the cells contacted with the compound are renal tubular cells and the activity measured is proliferation of said renal tubular cells.

9. The process of claim 4, wherein the cells contacted with the compound are renal tubular cells and the activity measured is transdifferentiation to myofibroblasts.

10. The process of claim 4, wherein the cells in the contacting step (i) have previously been transfected by the Ax1 gene.

11. The process of claim 10, wherein the transfected cells are either transiently or stably transfected.

12. The process of claim 4, wherein the controlled conditions in step (iii) comprises measurement upon contacting cells which lack an active Ax1 gene.

13. The process of claim 12, wherein the cells have a mutated inactive form of the Ax1 gene.

14. The process of claim 1, wherein the Ax1 receptor comprises consecutive amino acids, the sequence of which is set forth in SEQ ID NO:5.

15. The process of claim 14, wherein the Ax1 receptor comprises consecutive amino acids, the sequence of which is set forth in either SEQ ID NO:2 or SEQ ID NO:4.

16. The process of claim 14, wherein the Ax1 receptor comprises a biologically active portion of the intracellular domain.

17. The process of claim 1, wherein the compound inhibits the activity of the Ax1 receptor at least 2-fold more effectively than it inhibits the activity of the tyrosine kinase receptor FGFRI.

18. The process of claim 1, wherein the compound inhibits the activity of the Ax1 receptor at least 2-fold more effectively than it inhibits the activity of one or more of the set of tyrosine kinase receptors consisting of VER4, KIN24, HGFr, met, EGFR, IGF-1r, InsR and Ab1.

19. The process of claim 17, wherein the inhibition is at least 100-fold more effective.

20. Use of a compound identified according to the process of claim 1 in the preparation of a medicament for therapy of nephropathy.

21. The process of claim 1, wherein the receptor is contacted with the compound.

22. The process of claim 21, wherein the Ax1 receptor is immobilized.

23. The process of claim 21, wherein prior to step (i) an Ax1 receptor is contacted with a second compound known to bind Ax1.

24. The process of claim 23, wherein either the Ax1 receptor or the second compound are immobilized.

25. A process of preparing a composition which comprises:

(iii) identifying a compound that inhibits activity of a human Ax1 receptor using the process of claim 1; and

(iv) admixing said compound with a carrier.

26. The process of claim 25, wherein the carrier is a pharmaceutically effective carrier.

27. The process of claim 26, wherein the compound admixed with the carrier is present in a pharmaceutically effective amount.

28. A method of diagnosing nephropathy in a subject comprising determining in a sample from the subject the level of an Ax1 receptor polypeptide, wherein a higher level of the polypeptide compared to the level in a subject free of nephropathy is indicative of nephropathy.

29. The method of claim 28, wherein the Ax1 receptor comprises consecutive amino acids, the sequence of which is set forth in SEQ ID NO:5.

30. The process of claim 29, wherein the Ax1 receptor comprises consecutive amino acids, the sequence of which is set forth in either SEQ ID NO:2 or SEQ ID NO:4.

31. The method of claim 29, wherein the nephropathy is diabetic nephropathy.

32. The method of claim 31, wherein the nephropathy is kidney fibrosis.

33. The method of claim 28, wherein the sample is taken from a bodily fluid.

34. The method of claim 33, wherein the bodily fluid is blood or urine.

35. A process of identifying a compound capable of inhibiting the activity of a human Ax1 receptor by screening a plurality of compounds that comprises the steps of:

(i) contacting the Ax1 receptor or cells expressing the Ax1 receptor with the plurality of compounds;

(ii) measuring the Ax1 receptor activity in the presence of the plurality of compounds;

(iii) comparing the activity measured in step (ii) to that measured in the absence of the plurality of compounds under controlled conditions, wherein a decrease identifies the plurality of compounds as being capable of inhibiting the activity; and

(v) separately determining which compound or compounds present in the plurality inhibit the activity of a human Ax1 receptor.

* * * * *

X 3

Integrins and cancer

Judith A Varner* and David A Cheresh†

The past year or two has seen great advances in the elucidation of significant roles for integrins in cancer cells. These include roles in signal transduction, gene expression, proliferation, apoptosis regulation, invasion and metastasis, and angiogenesis. In particular, integrin $\alpha v\beta 3$ has been implicated in the neovascularization of tumors. In addition, this integrin has been shown to contribute to the survival, proliferation and metastatic phenotype of human melanoma.

Addresses
*Department of Medicine, University of California, San Diego, University Center 303, 9500 Gilman Drive, La Jolla, CA 92093-0063, USA; e-mail: jvarner@ucsd.edu
†Department of Immunology, The Scripps Research Institute, 10666 N Torrey Pines Rd, IMM 124, La Jolla, CA 92037, USA; e-mail: cheresh@scripps.edu

Current Opinion in Cell Biology 1996, 8:724–730

Abbreviations

bFGF	basic fibroblast growth factor
CAM	chorioallantoic membrane
FAK	focal adhesion kinase
MAPK	mitogen-activated protein kinase
RGD	Arg–Gly–Asp

Introduction

Although integrins were originally characterized as a family of cell surface receptors that are responsible for anchoring cells to the extracellular matrix, they have recently been shown to impact on such dynamic processes, in normal and tumor cells, as intracellular signaling and gene expression that leads to cell migration, proliferation, differentiation and survival. The integrin family is composed of 15 α and 8 β subunits that are contained in over twenty different $\alpha\beta$ heterodimeric combinations on cell surfaces. Integrins bind to extracellular matrix proteins or cell surface Ig family molecules through short peptide sequences present in the ligands. Although some integrins selectively recognize a single extracellular matrix protein ligand (e.g. $\alpha 5\beta 1$ integrin recognizes only fibronectin), others bind two or more ligands [1,2]. Several integrins recognize the tripeptide Arg–Gly–Asp (RGD) [1–3], whereas others recognize alternative short peptide sequences [1]. Combinations of different integrins on cell surfaces allow cells to recognize and respond to a variety of different extracellular matrix proteins.

Integrins mediate cellular adhesion to, and migration on, the extracellular matrix proteins found in intercellular spaces and basement membranes [1,2], but they also regulate cellular entry into, and withdrawal from, the cell cycle [4,5•,6]. Ligation of integrins by their extracellular matrix protein ligands induces a cascade of intracellular signals [7] that include tyrosine phosphorylation of focal adhesion kinase, increases in intracellular Ca^{2+} levels, inositol lipid synthesis, synthesis of cyclins [4], and expression of immediate early genes [5•]. In contrast, prevention of integrin–ligand interactions suppresses cellular growth or induces apoptotic cell death [5•,8–10,11•]. Thus, integrins play roles in a number of cellular processes that impact on the development of tumors, including the regulation of proliferation and apoptosis, cellular motility and invasion, cell surface localization of metalloproteinases, and angiogenesis (or the development of the vasculature that is an essential feature of solid tumor cancers). This review will focus on several of the key recent findings implicating integrin function in tumor proliferation, invasion and angiogenesis.

Integrins mediate signal transduction

Integrin ligation regulates biological events such as the survival, motility and proliferation of normal and tumor cells. Central to the many roles that integrins play in cancer are integrin-mediated signal transduction processes. Integrins transduce signals across the membrane upon ligation either by substrates such as fibronectin or by cross-linking with anti-integrin antibodies [12–14]. Among the integrin-generated signals identified to date are increases in intracellular pH [13–16], intracellular calcium [17–19], inositol lipid synthesis [20], and tyrosine phosphorylation of a tyrosine kinase associated with focal contacts, pp125 FAK (focal adhesion kinase) [21,22], in addition to activation of p34/cdc2 [23] and cyclin A [4]. Recently, the integrin-mediated activation of protein kinase C [24], mitogen-activated protein kinase (MAPK) [25•,26,27], phosphatidylinositol 3-kinase [28,29], p21Ras [30], and NF-κB [31] has also been demonstrated. Many of these signaling events can be induced directly by cross-linking of integrins on cell surfaces using specific monoclonal antibodies, suggesting that integrins alone, without accessory molecules, are responsible for these events.

The role of integrins in tumor cell proliferation

Abnormal cellular growth is one of the hallmarks of all tumors. It is now known that defects in some of the molecules that regulate the cellular proliferation machinery are common in tumor cells. Although the regulation of cellular proliferation is a complex process which requires the activities of growth factor receptors, kinases, cyclins, transcription factors and other molecules, normal cells can be induced to withdraw from the cell cycle simply by placing them in suspension [6]. Integrins on tumor cells are now thought to play intricate roles in the progression of solid tumors. Normal diploid cells can be induced to withdraw from the cell cycle and to become

quiescent by maintenance in anchorage-independent conditions [6]. They are dependent on anchorage not only for growth [6], but also for survival [8,10,11•]. In contrast to normal cells, transformed cells are characterized by their anchorage-independent growth.

The anchorage-independent growth of tumor cells may result from a transformation-associated uncoupling of cell cycle dependence on signals that are transduced by integrin-mediated attachment to the substratum [4]. Adhesion proteins have been associated with the regulation of growth since fibronectin was first characterized as the large external transformation sensitive protein (LETS) because it is lost from the surface of transformed cells [32,33]. Some tumor cells lose their ability to attach to fibronectin after transformation [34]; this may be the result of a transformation-associated loss of the fibronectin receptor, integrin α5β1, from the cell surface [35] or, alternatively, could be caused by inactivation of the integrin α5β1 via a phosphorylation event [36].

Integrin α5β1 expression and tumor growth

A role for integrin α5β1 in the regulation of proliferation of tumor cells was initially suggested by a series of studies of tumor variants which overexpress α5β1. MG63 osteosarcoma cells [37,38] and K562 erythroleukemia cells [39] that were selected for an increased ability to attach to fibronectin exhibited a fivefold upregulation of α5β1 expression concomitant with significantly reduced anchorage-independent growth and tumorigenicity. The direct induction of tumor cell growth inhibition by integrin α5β1 expression was demonstrated when transfection of Chinese hamster ovary cells with the integrin α5 and β1 subunit genes resulted in cells that expressed 30-fold more α5β1 and showed a loss of tumorigenicity and reduced proliferation *in vitro* [40]. These results also suggested that the degree of growth inhibition is dependent on the level of α5β1 expression on the cell surface. In additional studies, loss of integrin α5β1 expression on Chinese hamster ovary cells led to enhanced tumorigenicity [41]. These findings document that integrin α5β1 is implicated in the growth regulation of tumor cells.

Recently, Varner *et al.* [5•] expressed the integrin α5β1 in HT29 colon carcinoma cells which normally lack α5β1. After being transfected with a cDNA encoding the α5 integrin subunit, these cells gained the ability to adhere to fibronectin. Interestingly, in the absence of a fibronectin substrate, expression of integrin α5β1 leads to a dominant-negative regulation of cellular proliferation [5•]. Integrin α5 transfected cells were either nontumorigenic or significantly less tumorigenic than control transfectants and parental tumor cells, and they proliferated at half the rate of control transfectants under anchorage-independent culture conditions. This growth suppression is associated with a failure to enter S phase, as monitored by thymidine incorporation into DNA, and with an upregulation of transcription of the growth arrest inducing gene *gas-1* [42,43] and a downregulation of transcription of the immediate early genes c-*fos*, c-*jun* and *jun B*. Ligation with fibronectin reverses the inhibition of proliferation, inhibits transcription of *gas-1* and induces transcription of the immediate early genes, in a tyrosine phosphorylation dependent manner [5•]. Although no studies have indicated a role for other fibronectin receptors in the negative regulation of tumor growth, it remains unclear whether or not alternative fibronectin receptors could suppress tumor cell proliferation or whether this is a unique property of the ectodomain and/or cytoplasmic tail regions of the α5β1 integrin.

Distinct integrins influence the biology of various tumor types

Expression of other integrin subunits, in particular α2β1 and αvβ3, also influences cellular proliferation and differentiation. The loss of expression of the integrin α2β1 in breast epithelial cells is correlated with the transformed phenotype [44•]. Antisense mRNA reduction of α2β1 levels in breast carcinoma cells induces a transformed phenotype [45]. In addition, the ectopic expression of integrin α2β1, a receptor for laminin and collagen, has been shown to suppress the growth of breast carcinoma cells and to induce their differentiation [44•]. Expression of this integrin altered the phenotype of poorly differentiated human and mouse breast carcinoma cells from a fibroblastoid, spindle-shaped phenotype to an epithelioid, polygonal-shaped, contact-inhibited phenotype. These transfected cells were then able to form glandular structures in three-dimensional matrices.

In addition, a novel alternatively spliced integrin β1 subunit, β1C, has recently been described [46••]. This molecule is a growth inhibitory subunit which prevents cell cycle progression [46••]. This progression is dependent on an amino acid sequence in its cytoplasmic domain that is located between amino acids 795 and 802 [47•].

In contrast, expression of some integrins positively regulates tumor cell proliferation. Expression of the integrin αvβ3 in metastatic, but not benign, melanomas [48,49] suggests a role for this integrin in the regulation of tumor proliferation. When melanoma cells were selected for loss of the αv integrin subunit, these cells exhibited significantly reduced proliferation and tumorigenicity which could be restored by re-expression of the integrin [50,51]. In further support of a role for αv integrin in tumor cell proliferation are studies in which antibody antagonists of the αv subunit prevented human melanoma tumor formation in nude mice [52].

Expression of the integrin subunits α6 and α3 is also associated with transformation and tumor progression. Integrin α3β1 is expressed in 82% of metastatic tumors [53]. Integrin α6 is expressed at increased levels in tumors of the head and neck [54], and in bladder cancer [55], lung

cancer [56] and colon carcinoma (JA Varner, unpublished data).

The molecular mechanisms by which these integrins regulate tumor cell growth are not clear at present, but it is likely that integrin signaling plays a central role in the process. Recently, a novel oncoprotein with tyrosine kinase activity that directly interacts with the integrin β1 cytoplasmic tail was described [57•]. The interactions of this kinase, called integrin-linked kinase-1 (ILK-1), and of other such signal transduction mediators may play important roles in integrin-regulated cellular proliferation. Thus, the pattern of integrin expression in the tumor cell is implicated in the enhanced proliferation that is a characteristic of tumor cells.

Regulation of apoptosis by integrins

Cellular attachment of epithelial, endothelial and some tumor cells to the extracellular matrix through integrins (or integrin cross-linking) promotes cell survival by inhibiting apoptosis, as determined by evaluation of DNA laddering, cellular morphology and presence of free 3′-hydroxyl groups [8–10,11•,58]. In fact, *de novo* expression of αvβ3 on human melanoma cells facilitated the increased survival of the cells in three-dimensional dermal collagen [9]. In addition, integrin ligation has been shown to regulate the expression of Bcl-2, a key regulatory component in the suppression of apoptosis [59•]. Ligation of integrin α5β1 in α5-transfected tumor cells (Chinese hamster ovary tumor cells), which exhibit reduced proliferation as compared with untransfected cells, prevented apoptosis by inducing Bcl-2 expression [60•]. Ligation of integrin αvβ3 in endothelial cells suppresses p53 activity, inhibits p21WAF1/CIP1 expression and increases the Bcl-2 : Bax ratio, promoting cell survival [59•–61•]. In contrast, blocking integrin αvβ3 ligation with integrin antagonists induced p53 activation and blocked Bcl-2 expression [60•]. Interestingly, expression of the β4 cytoplasmic domain in cells activates p21 and induces growth arrest [61•].

Integrins in invasion and motility

Integrins also contribute to cellular motility and metastasis. For example, the integrin α2β1, a collagen/laminin receptor, has been shown to impart metastatic abilities to some tumor cells [62]. Integrin αvβ3, the most promiscuous member of the integrin family, mediates cellular adhesion to vitronectin, fibronectin, fibrinogen, laminin, collagen, von Willibrand factor, osteopontin, and adenovirus penton base, among other proteins [63–65]. Expression of this integrin enables a given cell to adhere to, migrate on, or respond to almost any matrix protein it may encounter. This migratory capacity is dependent on an intact NPXY (single-letter code for amino acids) sequence present within the integrin β3 subunit cytoplasmic tail [66]. Tumor cells transfected with a β3 cDNA containing a mutated NPXY sequence are unable to metastasize, in contrast to tumor cells transfected with an intact β3 subunit [66]. This integrin is expressed on migratory cells such as metastatic melanoma cells [48], in which its expression correlates with a role in metastasis [66,67]. An additional αv integrin, the integrin αvβ5, also directs tumor cell motility, but unlike αvβ3-mediated motility, αvβ5-mediated motility is dependent on receptor tyrosine kinase activity [68] and NF-κB-mediated gene expression [31].

Recently, the association of integrins and matrix metalloproteinases (MMPs) has been described. Recent studies by Brooks *et al.* [69•] demonstrated that the collagenase MMP-2 binds directly to integrin αvβ3 and is thus localized, in a proteolytically active form, on the surface of invasive tumor cells or endothelial cells. This localization appears to provide migratory cells with coordinated matrix degradation and cellular motility, thus facilitating cellular invasion processes [69•]. Furthermore, an association between integrin α2β1 and the positive regulation of MMP-1 expression has also been recently described [70], as has an association between integrin α5β1 and α4β1 ligation and metalloproteinase expression [71].

Role of integrins in tumor angiogenesis

Perhaps the most significant of the physiological roles played by integrin αvβ3 in cancer is its critical role in the process of angiogenesis. Integrin αvβ3 is minimally, if at all, expressed on resting, or normal, blood vessels, but is significantly upregulated on vascular cells within human tumors [10,72] and in response to growth factors *in vitro* [73,74] and *in vivo* [72,75]. For example, basic fibroblast growth factor (bFGF), but not transforming growth factor-β or interferon-γ, markedly increases β3 mRNA levels and β3 protein surface expression in cultured human dermal microvascular endothelial cells [73,74]. bFGF and tumor necrosis factor-α stimulate αvβ3 expression on developing blood vessels in the chick chorioallantoic membrane (CAM) and on the rabbit cornea [72,75]. Peak levels of integrin expression are observed on blood vessels 12–24 hours after stimulation with bFGF (our unpublished data). αvβ3 expression is also induced by human tumors cultured on the chick CAM [72,75] and by human tumors grown in human skin explants grafted onto SCID mice [76].

Antagonists of αvβ3 integrin promote tumor regression by disrupting angiogenesis

The highly restricted expression of αvβ3 integrin and the upregulation of its expression during angiogenesis suggest that it may play a critical role in the angiogenic process. In fact, recent experimental evidence supports this notion. Specifically, antagonists of integrin αvβ3, but not of β1 integrins, potently inhibit angiogenesis in a number of animal models. When angiogenesis is induced on the chick CAM with purified cytokines, αvβ3 expression is stimulated by fourfold within 72 hours [72]. Topical or systemic administration of LM609, a monoclonal antibody antagonist of αvβ3, inhibited angiogenesis, whereas other anti-integrin antibodies were ineffective [72]. Similarly, administration of LM609 or of a cyclic RGD peptide of

$\alpha v\beta 3$ antagonists, but not of other anti-integrin antibodies or of control peptides, reduced the growth of blood vessels into tumors growing on the surface of CAMs. Importantly, LM609 had no effect on pre-existing vessels [72]. These findings suggest that $\alpha v\beta 3$ plays a biological role in a critical event of blood vessel formation during tumor angiogenesis. Antagonists of integrin $\alpha v\beta 3$ not only prevent the growth of tumor-associated blood vessels but this results in the regression of established tumors *in vivo* [10]. Histological examination of the anti-$\alpha v\beta 3$-treated and control-treated tumors revealed that few, if any, viable tumor cells remained in the anti-$\alpha v\beta 3$ treated tumors [10]. In fact, these treated tumors contained no viable blood vessels.

It is important that antagonists of integrin $\alpha v\beta 3$ also inhibit tumor growth in human skin. In studies of the effect of these antagonists on human angiogenesis, Brooks *et al.* [76] transplanted human neonatal foreskins onto SCID mice. After permitting the skin to heal, they were able to demonstrate that the majority of the blood vessels within the human skin were human in origin. Human breast cancer tumors ($\alpha v\beta 3$-negative) were established in the human skin transplants on these animals. Two weeks later, the mice were treated intravenously with LM609 or control antibodies. Tumor growth was either completely suppressed (in 8 out of 12 mice) or was significantly inhibited as compared with mice treated with a control antibody. Angiogenesis was significantly inhibited (by at least 75%) in the LM609-treated animals. Thus, LM609 appears to be effective in regulating the human angiogenic response to human tumors growing in a human tissue.

Importantly, not only did the LM609-treated animals contain smaller tumors but the tumours also appeared considerably less malignant than tumors in control animals; specifically, their margins were well defined, showing no evidence of tumor cell invasion [76]. In addition, there were fewer proliferative tumor cells in the LM609-treated animals. This was associated with a sharp decrease in the blood vessel counts in these tumors. Thus, by blocking tumor-induced angiogenesis it was possible to curtail the invasive or malignant properties of the tumor.

$\alpha v\beta 3$ integrin regulates vascular cell survival *in vivo*

The mechanism of action of $\alpha v\beta 3$ antagonists in blocking angiogenesis appears to be related to their ability to selectively promote unscheduled programmed cell death (apoptosis) of newly sprouting blood vessels, on the basis of increased DNA laddering and ApopTag staining for the presence of free 3′-hydroxyl groups in tissues treated with integrin $\alpha v\beta 3$ antagonists [10]. To further evaluate the effects of these antagonists on vascular cell events, single-cell suspensions were prepared from CAMs treated with bFGF and in the presence or absence of LM609. These cells were then stained with the DNA dye propidium iodide to examine the DNA content per cell. Cells with greater than one copy of DNA were presumed to have entered the cell cycle. These cells were then costained with ApopTag to evaluate their degree of DNA breakdown. This costaining procedure revealed that bFGF could promote cell entry into the cell cycle and that LM609 caused ApopTag staining of these same cells. These findings demonstrated that the monoclonal antibody LM609 was capable of inducing apoptosis of vascular cells that had already responded to the cytokine [10], suggesting that $\alpha v\beta 3$ promotes a survival signal critical for cells completing the cell cycle.

More importantly, these findings demonstrate that antagonists of $\alpha v\beta 3$ integrin disrupt a stage of angiogenesis that occurs after induction but prior to vessel maturation. This is consistent with the studies by Drake *et al.* [77] showing that antagonists of $\alpha v\beta 3$ integrin blocked late-stage development of new blood vessels in the quail by preventing lumen formation. Together, these findings are consistent with the notion that $\alpha v\beta 3$ provides a survival signal to proliferative vascular cells during new blood vessel growth. Presumably, after new blood vessels are fully mature, the vascular cells are refractory to antagonists of this integrin. These findings may explain why antagonists of $\alpha v\beta 3$ selectively impact newly growing blood vessels. It is not currently known if integrin $\alpha v\beta 5$ antagonists also induce apoptosis in angiogenic blood vessels.

Angiogenesis depends both upon the stimulation of quiescent vascular cells by growth factors released from tumors or other diseased tissues and also upon the interaction of the integrins $\alpha v\beta 3$ and $\alpha v\beta 5$ with one of their ligands [72,75]. Stimulated endothelial cells depend on integrin function for survival during a critical period of the angiogenic process, as inhibition of $\alpha v\beta 3$–ligand interaction by antibody or peptide antagonists induces vascular cell apoptosis and inhibits angiogenesis [72,75].

Conclusions

Recent published reports have documented a significant role for integrins in the regulation of tumor cell survival, proliferation and invasion. Importantly, tumor cell growth and malignant behavior also depend on angiogenesis, a process that depends on the endothelial cell $\alpha v\beta 3$ integrin.

Future studies are likely to focus on integrin-mediated signaling and cell biological events that contribute to the malignant behavior of solid tumors.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Hynes RO: **Integrins: versatility, modulation and signaling in cell adhesion.** *Cell* 1992, **69**:11–25.

2. Cheresh D: **Integrins: structure, function and biological properties.** *Adv Mol Cell Biol* 1993, **6**:225–252.

3. Ruoslahti E, Pierschbacher M: **Arg–Gly–Asp: a versatile cell recognition sequence.** *Cell* 1986, **44**:517–518.

4. Guadagno TM, Ohtsubo M, Roberts JM, Assoian RK: **A link between cyclin A expression and adhesion-dependent cell cycle proliferation.** *Science* 1993, **262**:1572–1575.

5. • Varner JA, Emerson DA, Juliano RL: **Integrin α5β3 expression negatively regulates cell growth: reversal by attachment to fibronectin.** *Mol Biol Cell* 1995, **6**:725–740.

This paper present studies that characterize the molecular mechanisms behind the growth inhibitory properties of the integrin α5β1. Expression of the unligated integrin affects cellular proliferation by inhibiting immediate early gene transcription and inducing transcription of a growth inhibitory gene.

6. Dike LE, Farmer SR: **Cell adhesion induces expression of growth-associated genes in suspension arrested fibroblasts.** *Proc Natl Acad Sci USA* 1988, **85**:6792–6796.

7. Juliano RL, Haskill S: **Signal transduction from the extracellular matrix.** *J Cell Biol* 1993, **120**:577–585.

8. Meredith JE Jr, Fazeli B, Schwartz MA: **The extracellular matrix as a cell survival factor.** *Mol Biol Cell* 1993, **4**:953–961.

9. Montgomery AMP, Reisfeld RA, Cheresh DA: **Integrin αvβ3 rescues melanoma from apoptosis in a three-dimensional dermal collagen.** *Proc Natl Acad Sci USA* 1994, **91**:8856–8860.

10. Brooks PC, Montgomery AMP, Rosenfeld M, Reisfeld RA, Hu T, Klier G, Cheresh DA: **Integrin αvβ3 antagonists promote tumor regression by inducing apoptosis of angiogenic blood vessels.** *Cell* 1994, **79**:1157–1164.

11. • Boudreau N, Sympson CJ, Werb Z, Bissell M: **Suppression of ICE and apoptosis in mammary epithelial cells by extracellular matrix.** *Science* 1995 , **267**:891–893.

This paper demonstrates that antagonists of β1 integrins or overexpression of the matrix-degrading protease stromelysin induce apoptosis in mammary epithelial cells that have been previously shown to be dependent on the basement membrane for differentiation into glandular structures. Interleukin-1β-converting enzyme (ICE) expression is induced in these circumstances and inhibitors of ICE inhibit apoptosis, suggesting that integrin ligation may transduce signals that suppress apoptosis by inhibiting ICE expression.

12. Werb Z, Tremble PM, Behrendtsen O, Crowley E, Damsky CH: **Signal transduction through the fibronectin receptor induces collagenase and stromelysin gene expression.** *J Cell Biol* 1989, **109**:877–889.

13. Schwartz MA, Both G, Lechene C: **Effect of cell spreading on cytoplasmic pH in normal and transformed fibroblasts.** *Proc Natl Acad Sci USA* 1989, **86**:4525–4529.

14. Schwartz MA, Cragoe EJ Jr, Lechene CP: **pH regulation in spread and round cells.** *J Biol Chem* 1990, **265**:1327–1332.

15. Schwartz MA, Lechene C, Ingber DE: **Insoluble fibronectin activates the Na/H antiporter by clustering and immobilizing integrin α5β1, independent of cell shape.** *Proc Natl Acad Sci USA* 1991 **88**:7849–7853.

16. Schwartz MA, Lechene C: **Adhesion is required for protein kinase C-dependent activation of the Na+/H+ antiporter by platelet-derived growth factor.** *Proc Natl Acad Sci USA* 1992, **89**:6138–6141.

17. Schwartz MA: **Spreading of human endothelial cells on fibronectin or vitronectin triggers elevation of intracellular free calcium.** *J Cell Biol* 1992, **120**:1003–1010.

18. Pelletier AJ, Bodary SC, Levinson AD: **Signal transduction by the platelet integrin αIIbβ3: induction of calcium oscillations required for protein-tyrosine phosphorylation and ligand-induced spreading of stably transfected cells.** *Mol Biol Cell* 1992, **3**:989–998.

19. Leavesley DI, Schwartz MA, Cheresh DA: **Integrin β1- and β3-mediated endothelial cell migration is triggered through distinct signaling mechanisms.** *J Cell Biol* 1993, **121**:163–170.

20. McNamee HP, Ingber DE, Schwartz MA: **Adhesion to fibronectin stimulates inositol lipid synthesis and enhances PDGF-induced inositol lipid breakdown.** *J Cell Biol* 1993, **121**:673–678.

21. Kornberg LJ, Earp HS, Turner CE, Prockop C, Juliano RL: **Signal transduction by integrins: increased protein tyrosine phosphorylation caused by clustering of β1 integrins.** *Proc Natl Acad Sci USA* 1991, **88**:8392–8395.

22. Guan JL, Shalloway D: **Regulation of focal adhesion-associated protein tyrosine kinase by both cellular adhesion and oncogenic transformation.** *Nature* 1992, **358**:690–692.

23. Symington BE: **Fibronectin receptor modulates cyclin dependent kinase activity.** *J Biol Chem* 1993, **267**:25744–25747.

24. Vuori K, Ruoslahti E: **Activation of protein kinase C precedes α5β1 integrin-mediated cell spreading on fibronectin.** *J Cell Biol* 1993, **268**:21459–21462.

25. • Morino N, Mimura T, Hamasaki K, Tobe K, Ueki K, Kikuchi K, Takehara K, Kadowaki T, Yazaki Y, Nojima Y: **Matrix/integrin interaction activates the mitogen-activated protein kinases, p44erk-1 and p42erk-2.** *J Biol Chem* 1995, **270**:269–273.

This paper is one of the first to show that integrin ligation by attachment to the extracellular matrix protein fibronectin or by cross-linking with anti-integrin-β1 antibodies induces activation of the MAPKs extracellular regulated kinase (ERK)1 and ERK2. Furthermore, the authors show that this process is dependent on the actin cytoskeleton, as cytochalasin D inhibits the process.

26. Schlaepfer DD, Hanks SK, Hunter T, Van der Geer P: **Integrin mediated signal transduction linked to Ras pathway by Grb2 binding to focal adhesion kinase.** *Nature* 1994, **372**:786–790.

27. Chen Q, Kinch MS, Lin TH, Nurridge K, Juliano RL: **Integrin mediated cell adhesion activates mitogen activated protein kinases.** *J Biol Chem* 1994, **269**:26602–26605.

28. Chen H-C, Guan J-L: **Association of focal adhesion kinase with its potential substrate phosphatidylinositol 3 kinase.** *Proc Natl Acad Sci USA* 1994, **91**:10148–10152.

29. Guinebault C, Payrastre B, Racaud-Sultan C, Mazarguil H, Breton M, Maunco G, Plantavid M, Chap H: **Integrin-dependent translocation of phosphoinositide 3-kinase to the cytoskeleton of thrombin-activated platelets involves specific interaction of p85a with actin filamaents and focal adhesion kinase.** *J Cell Biol* 1995, **129**:831–842.

30. Kapron-Bras C, Fitz-Gibbon L, Jeevaratnam P, Wilkins J, Dedhar S: **Integrin clustering stimulates p21ras activation.** *J Biol Chem* 1993, **268**:20701–20704.

31. Yebra M, Filardo E, Bayna E, Kawahara J, Cheresh D: **Induction of carcinoma cell migration on vitronectin by NF-κB dependent gene expression.** *Mol Biol Cell* 1995, **6**:841–850.

32. Vaheri A, Ruoslahti E: **Fibroblast surface antigen produced but not retained by virus-transformed human cells.** *J Exp Med* 1975, 142:530–535.

33. Mautner V, Hynes RO: **Surface distribution of LETS protein in relation to the cytoskeleton of normal and transformed cells.** *J Cell Biol* 1977, **75**:743–768.

34. Wagner D, Ivatt R, Destree A, Hynes R: **Similarities and differences between fibronectins of normal and transformed hamster cells.** *J Biol Chem* 1981, **25**:11708–11715.

35. Plantefaber L, Hynes RO: **Changes in integrin receptors on oncogenically transformed cells.** *Cell* 1989, **56**:281–290.

36. Hirst R, Horwitz AF, Buck C, Rohrschneider L: **Phosphorylation of the fibronectin receptor complex in cells transformed by**

oncogenes that encode tyrosine kinases. *Proc Natl Acad Sci USA* 1986, **83**:6470–6474.

37. Dedhar S, Argraves WS, Suzuki S, Ruoslahti E, Pierschbacher MD: **Human osteosarcoma cells resistant to detachment by an Arg–Gly–Asp-containing peptide overproduce the fibronectin receptor.** *J Cell Biol* 1987, **105**:1175–1182.

38. Dedhar S, Mitchell MD, Pierschbacher MD: **The osteoblast-like differentiation of a variant of MG-63 osteosarcoma cell line correlated with altered adhesive properties.** *Connect Tissue Res* 1989, **20**:49–61.

39. Symington BE: **Fibronectin receptor overexpression and loss of transformed phenotype in a stable variant of the K562 cell line.** *Cell Regul* 1990, **1**:637–648.

40. Giancotti FG, Ruoslahti E: **Elevated levels of the α5β1 receptor suppresses the transformed phenotype of Chinese hamster ovary cells.** *Cell* 1990, **60**:849–859.

41. Schreiner C, Fisher M, Hussein S, Juliano RL: **Increased tumorigenicity of fibronectin receptor deficient Chinese hamster ovary cell variants.** *Cancer Res* 1991, **51**:1738–1740.

42. Del Sal G, Ruaro ME, Philipson L, Schneider C: **The growth arrest-specific gene, gas-1, is involved in growth suppression.** *Cell* 1992, **70**:595–607.

43. Del Sal G, Collavin L, Ruaro M, Edomi P, Saccone S, Valle GD, Schneider C: **Structure, function, and chromosome mapping of the growth suppressing human homologue of the murine gas-1 gene.** *Proc Natl Acad Sci USA* 1994, **91**:1848–1852.

44. • Zutter MM, Santoro SA, Staatz WD, Tsung YL: **Re-expression of the α2β1 integrin abrogates the malignant phenotype of breast carcinoma cells.** *Proc Natl Acad Sci USA* 1995, **92**:7411–7415.

During the transition of cells from the normal to the neoplastic state, expression of the integrin α2β1 is diminished. This paper demonstrates that re-expression of the α2β1 integrin in breast carcinoma cells dominantly regulates breast carcinoma cell behavior, restoring a more normal, glandular phenotype to breast carcinoma cells and inhibiting tumor development.

45. Keely PJ, Fong AM, Zutter MM, Santoro SA: **Alteration of collagen-dependent adhesion, motility and morphogenesis by the expression of antisense alpha 2 integrin in mRNA in mammary cells.** *J Cell Sci* 1995, **108**:595–607.

46. •• Meredith J, Takada Y, Fornaro M, Languino LR, Schwartz MA: **Inhibition of cell cycle progression by the alternatively spliced integrin beta (1C).** *Science* 1995, **269**:1570–1572.

This paper is a significant work that indicates that alternatively spliced integrins differing in cytoplasmic tail residues can impact cell behavior differentially. This is the first indication that a specific integrin cytoplasmic tail can dominantly regulate cell cycle progression.

47. • Fornaro M, Zheng DQ, Languino LR: **The novel structural motif Gln (795)–Gln (802) in the integrin beta 1C cytoplasmic domain regulates cell proliferation.** *J Biol Chem* 1995, **270**:24666–24669.

This paper demonstrates that a small region of the β1C integrin cytoplasmic tail is responsible for regulating the effects of β1C on cell cycle progression and cellular proliferation.

48. Albelda SM, Mette SA, Elder DE, Stewart R, Damjanovich L, Herlyn M, Buck CA: **Integrin distribution in malignant melanoma: association of the β3 subunit with tumor progression.** *Cancer Res* 1990, **50**:6757–6764.

49. Danen E, Jansen F, Van Kraats A, Cornelissen I, Ruiter D, Van Miujen G: **αv integrins in human melanoma: gain of αvβ3 and loss of αvβ5 are related to tumor progression *in situ* but not to metastatic capacity of cell lines in nude mice.** *Int J Cancer* 1995, **61**:491–496.

50. Felding-Habermann B, Mueller BM, Romerdahl CA, Cheresh DA: **Involvement of integrin αv gene expression in human melanoma.** *J Clin Invest* 1992, **89**:2018–2022.

51. Sanders LC, Felding-Habermann B, Mueller BM, Cheresh DA: **Role of αv integrins and vitronectin in human melanoma cell growth.** *Cold Spring Harb Symp Quant Biol* 1992, **58**:233–240.

52. Mitjans F, Sander D, Adan J, Sutter A, Martinez JM, Jaggle CS, Moyano JM, Kreysch HG, Piulats J, Goodman SL: **An anti-alpha v-integrin antibody that blocks integrin function inhibits the development of a human melanoma in nude mice.** *J Cell Sci* 1995, **108**:3067–3078.

53. Bartolazzi A, Cerboni C, Nicotra MR, Mottolese M, Bigotti A, Natali PG: **Transformation and tumor progression are frequently associated with expression of the alpha 3/beta 1 heterodimer in solid tumors.** *Int J Cancer* 1994, **58**:488–491.

54. Van Waes C, Carey TE: **Overexpression of the A9 antigen α6β4 in head and neck cancer.** *Otolaryngol Clin North Am* 1992, **25**:1117–1139.

55. Liebert M, Wedemeyer G, Stein JA, Washington RW, Van Waes C, Carey T, Grossman HB: **The monoclonal antibody BQ16 identifies the α6β4 integrin on bladder cancer.** *Hybridoma* 1993, **12**:67–80.

56. Costatini RM, Falcioni R, Battista P, Zupi G, Kennel S, Colasante A, Venturo I, Cucio C, Sacci A: **Integrin α6β4 expression in human lung cancer as monitored by specific monoclonal antibodies.** *Cancer Res* 1990, **50**:6107–6112.

57. • Hannigan GE, Leung-Hagesteijn C, Fitz-Gibbon L, Coppolino M, Radeva G, Bell JC, Dedhar S: **A novel, ankyrin repeat-containing serine/threonine protein kinase interacts with the β1 cytoplasmic domain.** *Nature* 1995, **379**:91–96.

This paper describes a novel integrin-associated serine/threonine kinase that can induce anchorage-independent growth and that can directly phosphorylate the integrin β1 cytoplasmic tail *in vitro*. This kinase may play an essential role in integrin-mediated signal transduction that contributes to induction of cellular proliferation.

58. Frisch SM, Francis H: **Disruption of epithelial cell–matrix interactions induces apoptosis.** *J Cell Biol* 1994, **124**:619–626.

59. • Zhang Z, Vuori K, Reed JC, Ruoslahti E: **The α5β1 integrin supports survival of cells on fibronectin and upregulates bcl-2 expression.** *Mol Biol Cell* 1995, **6**:841–850.

This paper is the first to document a regulation of the apoptosis suppressor Bcl-2 by integrin ligation. These studies demonstrate that integrins contribute to cell survival by transducing a signal that leads to Bcl-2 expression and function.

60. • Stromblad S, Becker J, Yebra M, Brooks P, Cheresh DA: **Suppression of p53 activity and p21 WAF1/CIP1 expression by vascular cell integrin αvβ3 during angiogenesis.** *J Clin Invest* 1996, **98**:426–433.

This paper documents the molecular mechanism by which antagonists of integrin αvβ3 inhibit angiogenesis by promoting apoptosis of endothelial cells. These studies demonstrate a regulation of p53 activity and Bcl-2 expression by integrin ligation that is inhibited by αvβ3 antagonists.

61. • Clarke AS, Lotz MM, Chao C, Mercurio AM: **Activation of the p21 pathway of growth arrest and apoptosis by the beta (4) integrin cytoplasmic domain.** *J Biol Chem* 1995, **270**:22673–22676.

This paper demonstrates that the integrin β4 subunit induces cell cycle arrest in colon carcinoma cells by inducing the expression of the cell cycle inhibitor p21 WAF1/CIP1, and that the cytoplasmic tail of integrin β4 is required for this activity.

62. Chan BM, Chan C, Matsuura, N, Takada Y, Zetter BR, Hemler ME: ***In vitro* and *in vivo* consequences of VLA-2 expression on rhabdomyosarcoma cells.** *Science* 1992, **251**:1600–1602.

63. Cheresh D: **Human endothelial cells synthesize and express an Arg–Gly–Asp directed adhesion receptor involved in attachment to fibrinogen and von Willebrand factor.** *Proc Natl Acad Sci USA* 1987, **84**:6471–6475.

64. Leavesley DI, Ferguson GD, Wayner EA, Cheresh DA: **Requirement of integrin β3 subunit for carcinoma cell spreading or migration on vitronectin and fibrinogen.** *J Cell Biol* 1992, **117**:1101–1107.

65. Wickham TJ, Mathias P, Cheresh DA, Nemerow GR: **Integrins αvβ3 and αvβ5 promote adenovirus internalization but not virus attachment.** *Cell* 1993, 73:309–319.

66. Filardo EJ, Brooks PC, Deming SL, Cheresh DA: **Requirement of the NPXY motif in the integrin β3 subunit cytoplasmic tail for melanoma cell migration *in vitro* and *in vivo*.** *J Cell Biol* 1995, 130:441–450.

67. Nip J, Shibata H, Loskutoff D, Cheresh D, Brodt P: **Human melanoma cells derived from lymphatic metastases use integrin αvβ3 to adhere to lymph node vitronectin.** *J Clin Invest* 1992, 90:1406–1413.

68. Klemke RL, Yebra M, Bayna EM, Cheresh DA: **Receptor tyrosine kinase signaling required for integrin αvβ5 directed cell motility but not adhesion on vitronectin.** *J Cell Biol* 1994, 127:859–866.

69. • Brooks PC, Stromblad S, Sanders L, Von Schalscha T, Aimes RT, Stetler-Stevenson WG, Quigley JP, Cheresh D: **Localization of matrix metalloproteinase MMP-2 to the surface of invasive cells by interaction with integrin αvβ3.** *Cell* 1996, 85:683–693.
This paper is the first documentation that integrins and matrix metalloproteinases directly interact to influence tumor and endothelial cell invasion processes. These studies demonstrate that activated matrix metalloproteinase 2 binds to integrin αvβ3, and that this interaction promotes cellular invasion and contributes to angiogenesis.

70. Riikonen T, Westermarck J, Koivisto L, Broberg A, Kahari VM, Heino, J. **Integrin alpha 2 beta 1 is a positive regulator of collagenase (MMP-1) and collagen alpha 1 (I) gene expression.** *J Biol Chem* 1995, 270:13548–13552.

71. Huhtala P, Humphries MJ, Mccarthy JB, Tremble PM, Werb Z, Damsky CH: **Cooperative signalling by alpha 5 beta 1 and alpha 4 beta 1 integrins regulates metalloproteinase gene expression in fibroblasts adhering to fibronectin.** *J Cell Biol* 1995, 129:867–879.

72. Brooks PC, Clark RAF, Cheresh DA: **Requirement of vascular integrin αvβ3 for angiogenesis.** *Science* 1994, 264:569–571.

73. Sepp NT, Li L-J, Lee KH, Brown EJ, Caughman SWW, Lawley TJ, Swerlick RA: **Basic fibroblast growth factor increases expression of the αvβ3 complex on human microvessel endothelial cells.** *J Invest Dermatol* 1994, 103:295–299.

74. Enenstein J, Walsh NS, Kramer RH: **Basic FGF and TGFβ differentially modulate integrin expression of human microvascular endothelial cells.** *Exp Cell Res* 1992, 203:499–503.

75. Friedlander M, Brooks PC, Shaffer RW, Kincaid CM, Varner JA, Cheresh DA: **Definition of two angiogenic pathways by distinct αv integrins.** *Science* 1995, 27:1500–1502.

76. Brooks P, Stromblad S, Klemke R, Visscher D, Sarkar F, Cheresh D: **Antiintegrin αvβ3 blocks breast cancer growth and angiogenesis in human skin.** *J Clin Invest* 1995, 96:1815–1822.

77. Drake CJ, Cheresh DA, Little CD: **An antagonist of integrin αvβ3 prevents maturation of blood vessels during embryonic neovascularization.** *J Cell Sci* 1995, 108:2655–2661.

COPY

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/696,909	10/29/2003	James B. Lorens	7946-79836-01	9257

74839 7590 03/18/2010
Klarquist Sparkman, LLP
121 SW Salmon St
Suite 1600
Portland, OR 97204

EXAMINER	
REDDIG, PETER J	

ART UNIT	PAPER NUMBER
1642	

NOTIFICATION DATE	DELIVERY MODE
03/18/2010	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

tanya.harding@klarquist.com
docketing@klarquist.com

COPY

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

Ex parte JAMES B. LORENS, ROBERT E. ATCHISON,
ANNABELLE FRIERA, and SACHA HOLLAND

Appeal 2009-011194[1]
Application 10/696,909
Technology Center 1600

Decided: March 16, 2010

Before RICHARD M. LEBOVITZ, FRANCISCO C. PRATS, and JEFFREY N. FREDMAN, *Administrative Patent Judges*.

PRATS, *Administrative Patent Judge*.

DECISION ON APPEAL

This appeal under 35 U.S.C. § 134 involves claims to methods for identifying compounds that inhibit angiogenesis. The Examiner rejected the claims as anticipated and obvious.

---

[1] Rigel Pharmaceuticals, Inc., is the real party in interest (App. Br. 2).

We have jurisdiction under 35 U.S.C. § 6(b). We affirm the anticipation rejection but reverse the obviousness rejection.

STATEMENT OF THE CASE

Claims 1, 12, 14-18, 27, 41-44, and 54-61 are pending and on appeal (App. Br. 2). Claims 1, 27, and 56 are representative of the appealed subject matter and read as follows:

> 1. A method for identifying a compound that inhibits angiogenesis, the method comprising:
>
> assaying *in vitro* kinase activity of an Axl polypeptide comprising an amino acid sequence with greater than about 95% identity to full length SEQ ID NO: 4 in the presence of the compound, wherein the Axl polypeptide has kinase activity in the absence of said compound; and
>
> performing a cell-based assay in an endothelial cell comprising said Axl polypeptide in the presence of the compound, which assay produces an angiogenesis phenotype in said endothelial cell in the absence of the compound,
>
> wherein inhibition of the *in vitro* kinase activity of the Axl polypeptide in the presence of the compound and inhibition of the angiogenesis phenotype in the cell-based assay in the presence of the compound identifies the compound as a compound that inhibits angiogenesis.
>
> 27. An *in vitro* method for identifying a compound that inhibits angiogenesis, the method comprising:
>
> contacting the compound with an endothelial cell that expresses a recombinant Axl polypeptide comprising an amino acid sequence with greater than about 95% identity to full length SEQ ID NO: 4, wherein the Axl polypeptide has kinase activity in the absence of said compound; and
>
> performing a cell-based assay, which assay produces an angiogenesis phenotype in said endothelial cell in the absence of the compound,
>
> wherein inhibition of the angiogenesis phenotype in the cell-based assay in the presence of the compound identifies the compound as a compound that inhibits angiogenesis.

56. A method for identifying a compound that inhibits angiogenesis, the method comprising:

contacting the compound with a cell expressing a recombinant Axl polypeptide comprising an amino acid sequence with greater than about 95% identity to full length SEQ ID NO: 4, wherein the Axl polypeptide has kinase activity in the absence of said compound; and

assaying the kinase activity of the Axl polypeptide,

wherein inhibition of the kinase activity of the Axl polypeptide in the presence of the compound identifies the compound as a compound that inhibits angiogenesis.

The Examiner cites the following documents as evidence of unpatentability:

Ruoslahti	US 6,180,084 B1	Jan. 30, 2001
Panzer	US 2004/0048253 A1	Mar. 11, 2004
Klinghoffer	US 2004/0077574 A1	Apr. 22, 2004

Aileen M. Healy et al., *Gas 6 promotes Axl-mediated survival in pulmonary endothelial cells*, 280 AM. J. PHYSIOL. CELL MOL. PHYSIOL. L1273-L1281 (2001).

Judith A. Varner et al., *Integrins and cancer*, 8 CURRENT OPINION IN CELL BIOLOGY 724-730 (1996).

The following rejections are before us for review:[2]

(1) Claims 1, 14, 27, 54-56, and 61, rejected under 35 U.S.C. § 102(b) as being anticipated by Healy (Ans. 3-6); and

(2) Claims 12, 15-18, 41-44, and 57-60, rejected under 35 U.S.C. § 103(a) as being unpatentable over Healy as applied to claims 1, 14, 27, 54-56 and 61, in view of Varner, Ruoslahti, Panzer, and Klinghoffer (Ans. 6-7).

---

[2] The Examiner withdrew the appealed rejections under 35 U.S.C. § 112, first and second paragraphs (Ans. 2).

ANTICIPATION

ISSUE

The Examiner finds that Healy discloses "determining the in vitro kinase activity of an Axl polypeptide where the Axl polypeptide has kinase activity in the absence of the compound, see Fig. 5 and page L1276, 2nd col." (Ans. 4).

The Examiner further finds that Healy discloses "performing a cell-based assay in an endothelial cell by contacting human pulmonary endothelial cells that express human Axl (see Fig. 2) with the Axl ligand Gas 6 and determining the effect of this interaction on cell number, see Abstract, p. 1276, left column, and Fig. 6" (*id.*). The Examiner also notes that Healy "teaches assaying apoptosis in human endothelial cells expressing recombinant wild type Axl, see p. L1278 and Figure 9 and 10" (*id.*).

The Examiner further notes that "a wherein clause in a method claim is not given weight when it simply expresses the intended result of a process step positively recited, MPEP [§] 2111.04" (*id.*). Therefore, the Examiner reasons, "[g]iven that the method of the prior art comprises the same method steps as claimed in the instant invention, . . . the claimed method is anticipated because the method will inherently be a method for identifying a compound that inhibits angiogenesis" (*id.* at 4-5).

Appellants contend that, although Healy discloses that "contacting human pulmonary artery endothelial cells (HPAEC) which express Axl polypeptide, with exogenous Gas 6 (an Axl ligand) increased Axl phosphorylation . . ., increased cell number . . ., and decreased apoptosis of the cells in serum free medium . . ., these assays are all described independently" (App. Br. 16). Thus, Appellants argue, Healy does not

"teach the *combination* of assaying *in vitro* kinase activity of an Axl polypeptide in the presence of a test compound *and* performing a cell-based assay in the presence of the compound which produces an angiogenesis phenotype in the absence of the test compound, as in claim 1 (*id.* at 15-16).

Moreover, Appellants argue, Healy does not "teach that Gas 6 (an Axl polypeptide agonist) is an angiogenesis inhibitor" (*id.* at 16). Appellants cite the Gallichio[3] reference in support of this assertion (*id.* at 17). Appellants argue that Healy therefore "does not anticipate any of the claims (including independent claims 1, 27, and 56 and any claims that depend from these claims)" (*id.*; *see also* App. Br. 18-19).

Appellants further argue that the independent claims' preambles should be given patentable weight beyond merely reciting an intended purpose of the claimed method "as there has been clear reliance on the preamble to distinguish the claimed invention from Healy *et al.* throughout the prosecution history" (Reply Br. 3).

Appellants also argue that the "wherein" clauses in the independent claims "must be given patentable weight" because, "in order to achieve the claimed invention, one must determine whether the tested compound is an inhibitor of angiogenesis (as opposed to a compound that has no effect on angiogenesis or stimulates angiogenesis)" (*id.* at 4). Thus, Appellants argue,

> [t]his determination is expressed in the wherein clause, such that if the compound inhibits the kinase activity and/or the angiogenesis phenotype in the cell-based assay, then the compound is identified as an inhibitor of angiogenesis (see,

---

[3] Margherita Gallichio et al., *Inhibition of vascular endothelial growth factor receptor 2-mediated endothelial cell activation by Axl tyrosine kinase receptor*, 105 BLOOD 1970-76 (2005).

> e.g., specification at page 9, lines 16-22; page 30, lines 6-10). Without the wherein clause, one does not in fact achieve the identification of a compound that inhibits angiogenesis.

(*Id.*).

In view of the positions advanced by Appellants and the Examiner, the issue with respect to this rejection is whether Healy discloses a process encompassed by independent claims 1, 27, and 56.

FINDINGS OF FACT ("FF")

1. Healy discloses that "Gas 6, the product of the growth arrest-specific gene 6, is a soluble factor implicated in the regulation of multiple cellular functions, including growth, survival, adhesion, and chemotaxis" (Healy L1273 (citations omitted)).

2. Healy investigated "whether Gas 6 regulates endothelial cell survival at growth arrest. To address this question, we characterized Axl, Rse, and Gas 6 expression in human pulmonary artery endothelial cells (HPAEC)" (*id.* at L1274).

3. Healy "found that the Axl receptor is phosphorylated in untreated cells (Fig. 5, lane 1). Moreover, the addition of exogenous Gas 6 (Fig. 5, lane 2) but not of serum (Fig. 5, lane 3) or protein S (data not shown) enhances Axl phosphorylation 3.5-fold" (*id.* at L1276).

4. Healy discloses that "[o]ur data show that the addition of recombinant human Gas 6 to HPAEC cultures results in a statistically significant increase in cell number (Fig. 6)" (*id.*).

5. Healy discloses "results suggest[ing] that both the endogenous and exogenous Gas 6 function to inhibit HPAEC programmed cell death" (*id.* at L1278).

6. Healy also discloses that, when HPAEC cells expressing exogenous Axl were tested, "Gas 6 decrease[d] the number of apoptotic . . . HPAEC by 54%" (*id.*).

PRINCIPLES OF LAW

"To anticipate a claim, a prior art reference must disclose every limitation of the claimed invention, either explicitly or inherently." *In re Schreiber*, 128 F.3d 1473, 1477 (Fed. Cir. 1997).

During examination, the PTO must interpret terms in a claim using "the broadest reasonable meaning of the words in their ordinary usage as they would be understood by one of ordinary skill in the art, taking into account whatever enlightenment by way of definitions or otherwise that may be afforded by the written description contained in the applicant's specification." *In re Morris*, 127 F.3d 1048, 1054 (Fed. Cir. 1997).

As stated in *In re Zletz*, 893 F.2d 319, 322 (Fed. Cir. 1989), the reason for this rule of interpretation is that "during patent prosecution when claims can be amended, ambiguities should be recognized, scope and breadth of language explored, and clarification imposed."

Moreover, "[a]bsent claim language carrying a narrow meaning, the PTO should only limit the claim based on the specification or prosecution history when those sources expressly disclaim the broader definition." *In re Bigio,* 381 F.3d 1320, 1325 (Fed Cir. 2004). Thus, "while it is true that claims are to be interpreted *in light of* the specification and with a view to ascertaining the invention, it does not follow that limitations from the specification may be read into the claims." *Sjolund v. Musland*, 847 F.2d 1573, 1581 (Fed. Cir. 1988).

Regarding process claims, a preamble recitation that merely expresses the purpose of performing the claimed steps is not a limitation on the process where the body of the claim fully sets forth the steps required to practice the claimed process, and where the preamble recitation does not affect the how the claimed steps are to be performed. *See Bristol-Myers Squibb Co. v. Ben Venue Labs., Inc.*, 246 F.3d 1368, 1375-76 (Fed. Cir. 2001).

Also, "[a] 'whereby' clause that merely states the result of the limitations in the claim adds nothing to the patentability or substance of the claim." *Texas Instruments, Inc. v. International Trade Comm.*, 988 F.2d 1165, 1172 (Fed. Cir. 1993).

ANALYSIS

Appellants' arguments do not persuade us that Healy fails to disclose a process encompassed by independent claims 1, 27, and 56.

First, we are not persuaded that those claims' preambles limit the claimed processes to include a positive step of actually identifying a compound that inhibits angiogenesis. For example, the preambles of claims 1 and 56 recite "[a] method *for* identifying a compound that inhibits angiogenesis" (emphasis added). The preamble of claim 27 similarly recites "[a]n *in vitro* method *for* identifying a compound that inhibits angiogenesis" (emphasis added).

Thus, by their terms, the preambles do not require identification of the compound. Rather, the preambles describe the purpose of performing the steps recited in the bodies of the claims. *Cf. Bristol-Myers Squibb v. Ben Venue Labs.*, 246 F.3d at 1375-76 (preamble reciting "method *for* treating cancer patient" (emphasis added) held not to limit claim because recitation "d[id] not result in a manipulative difference in the steps of the claim").

We acknowledge Appellants' argument that the preamble was amended to its current form with the intention of distinguishing over processes that do not identify angiogenesis inhibitors (*see* Reply Br. 3). In the instant case, however, Appellants' argument regarding the scope of the preambles conflicts with the actual language in the preambles. The words of the preambles suggest a purpose for the steps recited in the claims' bodies, rather than another step in an addition to the steps actively recited.

Thus, given the conflict between the preamble interpretation advanced by Appellants and the actual language at issue, we are not persuaded that Appellants' actions during prosecution are sufficient to unambiguously disclaim the plain meaning of the words in the preambles. Rather, as stated in *In re Zletz*, 893 F.2d at 322, "during patent prosecution when claims can be amended, ambiguities should be recognized, scope and breadth of language explored, and clarification imposed."

Accordingly, given the language in the preambles, and the fact that the claimed steps are performed in the same way whether or not the candidate compound is actually an angiogenesis inhibitor, we are not persuaded that the preambles limit the claimed processes. We therefore do not agree with Appellants that the preambles of claims 1, 27, and 56 should be interpreted as requiring a practitioner performing the claimed processes to actually identify a compound that inhibits angiogenesis.

Nor do we agree with Appellants that the "wherein" clauses in those claims recite a positive step of identifying an angiogenesis inhibitor. For example, claim 1 recites two positive process steps:

> [(a)] assaying *in vitro* kinase activity of an Axl polypeptide comprising an amino acid sequence with greater than about 95% identity to full length SEQ ID NO: 4 in the

> presence of the compound, wherein the Axl polypeptide has kinase activity in the absence of said compound; and
>
> [(b)] performing a cell-based assay in an endothelial cell comprising said Axl polypeptide in the presence of the compound, which assay produces an angiogenesis phenotype in said endothelial cell in the absence of the compound . . . .

Because of the active "assaying" and "performing" language used, it is clear that claim 1 requires a practitioner to perform those steps to be within the scope of the claim.

The wherein clause, however, does not include active language comparable to the active "assaying" and "performing" steps:

> wherein inhibition of the *in vitro* kinase activity of the Axl polypeptide in the presence of the compound and inhibition of the angiogenesis phenotype in the cell-based assay in the presence of the compound identifies the compound as a compound that inhibits angiogenesis.

Rather, given the language used, the wherein clause is reasonably interpreted to identify the conditions that need to be satisfied in order to identify a compound "as a compound that inhibits angiogenesis." Accordingly, while the claim requires that the assays be performed on a compound, there is no step in the claim that additionally requires the compound to have inhibited kinase activity or to have inhibited the angiogenesis phenotype. The "wherein" clause specifies that "inhibition of" the recited activity and phenotype identifies the compound as an inhibitor, but does not recite that a compound achieved a positive result by actually inhibiting the kinase and cell-based activities.

Thus, the wherein clauses at issue are akin to a "whereby" clause that merely states the result of the other features of the claim. As noted above, "[a] 'whereby' clause that merely states the result of the limitations in the claim adds nothing to the patentability or substance of the claim." *Texas Instruments v. International Trade Comm.*, 988 F.2d at 1172.

This interpretation squares with Appellants' arguments, which recognize that the wherein clause is conditional in nature: "[t]his determination [of whether a compound is an angiogenesis inhibitor] is expressed in the wherein clause, such that *if* the compound inhibits the kinase activity and/or the angiogenesis phenotype in the cell-based assay, *then* the compound is identified as an inhibitor of angiogenesis" (Reply Br. 4 (emphasis added)).

Thus, while it may be true that the Specification states what an inhibitor is, and also states that the disclosed assays can identify inhibitors (*id.* (citing Spec. 9:16-22 and 30:6-10)), given the conditional nature of the language used in the wherein clauses at issue, we are not persuaded that the wherein clauses require the practitioner to perform either an inhibiting step or an identifying step.

In sum, we agree with the Examiner that the preambles and the wherein clauses of claims 1, 27, and 56 do not require the compound tested in any of the claimed methods to inhibit either the *in vitro* kinase activity of the Axl polypeptide, or the angiogenesis phenotype.

Turning to Healy, we note, as Appellants argue, that the tested compound Gas 6 actually promotes Axl phosphorylation (i.e. kinase) activity rather than inhibits it (FF 3), and also increases cell numbers in culture (FF 4), and decreases apoptosis in cells expressing recombinant Axl (FF 5-6).

As Appellants also argue, it appears that these properties would *not* identify Gas 6 as an angiogenesis inhibitor according to claims 1, 27, and 56.

However, as discussed above, the claims do not require the tested compound to actually inhibit either the kinase or cell-based assays. Thus, the fact that the compound tested in Healy does not inhibit the claim-designated activities does not demonstrate a lack of anticipation.

Lastly, we note that Healy's phosphorylase (i.e. kinase) assay and cell based assays were conducted separately (*see* FF 1-5). However, we do not see, and Appellants do not point to, any recitation in claim 1 regarding the timing of the assays, much less a requirement that the assays be performed simultaneously. Thus, the fact that Healy studied the effects of Gas 6 on Axl expression in different assays performed at different times does not, in our view, demonstrate that Healy does not anticipate claim 1.

In sum, for the reasons discussed, we do not agree with Appellants that the preambles and wherein clauses of independent claims 1, 27, and 56 distinguish those claims from the processes described in Healy. Nor are we persuaded that those claims are otherwise distinguishable over Healy.

We therefore affirm the Examiner's rejection of claims 1, 27, and 56 as anticipated by Healy, as well as claims 14, 54, 55, and 61, which were not argued separately. *See* 37 C.F.R. § 41.37(c)(1)(vii).

OBVIOUSNESS

ISSUE

Claims 12, 15-18, 41-44, and 57-60, rejected under 35 U.S.C. § 103(a) as being unpatentable over Healy as applied to claims 1, 14, 27, 54-56 and 61, in view of Varner, Ruoslahti, Panzer, and Klinghoffer (Ans. 6-7).

The Examiner concedes that Healy does not "teach determining the functional effect by measuring αvβ3 expression or haptotaxis or the use of an antibody, an antisense molecule, an RNAi molecule, or a small organic molecule" (*id.* at 6), and cites Varner, Ruoslahti, Panzer, and Klinghoffer to meet those features (*id.* at 6-7). Based on the references' teachings, the Examiner reasons:

> It would have been *prima facie* obvious at the time the invention was made to perform the method of claim 1 by measuring αvβ3 expression and to use an antibody, antisense molecule, RNAi, or small organic molecule as the compound to use in the screening methods for claims 1, 27, and 56 because the level of αvβ3 expression was known to be important in angiogenesis and the screening of various modulatory compounds for therapeutic purposes was conventionally used in the art at the time of the invention and the advantages of siRNA over other sequence specific modulators was well known in the art at the time the invention was made.

(*Id.* at 7.)

Appellants contend that the Examiner did not "provide any rationale for one of skill in the art to combine or modify the cited references. Taken together, one of skill might be motivated to assay regulation of apoptosis by Axl, but not regulation of angiogenesis" (App. Br. 20). Moreover, Appellants argue "[w]ithout the recognition that inhibition of Axl inhibits angiogenesis, there is no motivation to combine the references and no expectation of success in arriving at Applicants' claimed invention by combining the references" (*id.* at 20-21).

In view of the positions advanced by Appellants and the Examiner, the issue with respect to this rejection is whether the evidence of record supports the Examiner's conclusion that an ordinary artisan would have

found claims 12, 15-18, 41-44, and 57-60 prima facie obvious in view of Healy, Varner, Ruoslahti, Panzer, and Klinghoffer.

FINDINGS OF FACT

7. Healy concludes its study by stating:

> Programmed cell death is an integral component of the vascular response to injury. On the one hand, apoptosis in vascular smooth muscle cells counters the exuberant cellular proliferation that leads to intimal thickening. On the other hand, apoptosis in vascular endothelium contributes to pathogenesis by promoting intravascular coagulation activation. *Apoptosis also has a role in the vascular remodeling associated with tumor angiogenesis.* Thus a balance between cell growth and cell death may be required for vascular remodeling. In this report, we characterized the expression and function of the Gas 6 signaling pathway in pulmonary endothelium in vitro. Further elucidation of this pathway will reveal whether Gas 6 functions in maintaining the equilibrium between cell growth and survival in lung endothelium in vivo.

(Healy L1280 (emphasis added).)

8. Varner is a review article that "focus[es] on several of the key recent findings implicating integrin function in tumor proliferation, invasion and angiogenesis" (Varner 724).

9. Varner discloses that "[p]erhaps the most significant of the physiological roles played by integrin $\alpha v\beta 3$ in cancer is its critical role in the process of angiogenesis" as evidenced by the fact that it is "minimally, if at all, expressed on resting, or normal, blood vessels, but is significantly upregulated on vascular cells within human tumors and in response to growth factors in vitro and in vivo" (*id.* at 726 (citations omitted)).

10. Panzer discloses "purified human polynucleotides for diagnostics and therapeutics (dithp). Also encompassed are the polypeptides (DITHP) encoded by dithp" (Panzer, abstract).

11. Panzer discloses:

> DITHP encoded by polynucleotides of the present invention may be used to screen for molecules that bind to or are bound by the encoded polypeptides. The binding of the polypeptide and the molecule may activate (agonist), increase, inhibit (antagonist), or decrease activity of the polypeptide or the bound molecule. Examples of such molecules include antibodies, oligonucleotides, proteins (e.g., receptors), or small molecules.

(*Id.* at [0735].)

12. Panzer also discloses that the polynucleotides of its invention "are useful in antisense technology" (*id.* at [0754]).

13. Ruoslahti discloses a method for identifying a "molecule that homes to angiogenic vasculature by contacting a substantially purified NGR receptor with one or more molecules and determining specific binding of a molecule to the NGR receptor, where the presence of specific binding identifies the molecule as a tumor homing molecule that homes to angiogenic vasculature" (Ruoslahti, abstract).

14. Ruoslahti discloses that its methods can be used to screen libraries of DNA molecules (*id.* at col. 10, ll. 37-55) as well as antibodies (*id.* at col. 11, ll. 25-37).

15. Klinghoffer discloses "[c] Compositions and methods relating to small interfering RNA (siRNA) polynucleotides are provided as pertains to modulation of biological signal transduction" (Klinghoffer, abstract).

16. Klinghoffer discloses:

> siRNA polynucleotides may offer certain advantages over other polynucleotides known to the art for use in sequence-specific alteration or modulation of gene expression to yield altered levels of an encoded polypeptide product. These advantages include lower effective siRNA polynucleotide concentrations, enhanced siRNA polynucleotide stability, and shorter siRNA polynucleotide oligonucleotide lengths relative to such other polynucleotides (e.g., antisense, ribozyme or triplex polynucleotides).

(*Id.* at [0025].)

PRINCIPLES OF LAW

In *KSR Int' l Co. v. Teleflex Inc.*, 550 U.S. 398, 415 (2007), the Supreme Court emphasized "an expansive and flexible approach" to the obviousness question. The Court also reaffirmed, however, that "a patent composed of several elements is not proved obvious merely by demonstrating that each of its elements was, independently, known in the prior art." *Id.* at 418.

Rather, as the Court stated:

> [I]t can be important to identify a reason that would have prompted a person of ordinary skill in the relevant field to combine the elements *in the way the claimed new invention does* . . . because inventions in most, if not all, instances rely upon building blocks long since uncovered, and claimed discoveries almost of necessity will be combinations of what, in some sense, is already known.

Id. at 418-419 (emphasis added).

Ultimately, therefore, as our reviewing court has stated, "[i]n determining whether obviousness is established by combining the teachings of the prior art, the test is what the combined teachings of the references

would have suggested to those of ordinary skill in the art." *In re GPAC Inc.*, 57 F.3d 1573, 1581 (Fed. Cir. 1995) (internal quotations omitted).

Moreover, "patents are not barred just because it was obvious 'to explore a new technology or general approach that seemed to be a promising field of experimentation, where the prior art gave only general guidance as to the particular form of the claimed invention or how to achieve it.'" *Procter & Gamble Co. v. Teva Pharmaceuticals USA, Inc.*, 566 F.3d 989, 997 (Fed. Cir. 2009) (quoting *In re O'Farrell*, 853 F.2d, 894, 903 (Fed. Cir. 1988)).

ANALYSIS

We agree with Appellants that the Examiner has not made a prima facie case of obviousness with respect to claims 12, 15-18, 41-44, and 57-60.

Claim 12 recites "[t]he method of claim 1, wherein the angiogenesis phenotype is αvβ3 expression, tube formation or haptotaxis." Thus, the "cell-based assay . . . which assay produces an angiogenesis phenotype" must be an assay which detects αvβ3 expression.

We acknowledge Healy's disclosure that apoptosis plays a role in tumor-related angiogenesis (FF 7). We also acknowledge Varner's disclosure that αvβ3 expression plays a significant role in tumor-related angiogenesis (FF 9).

However, Healy's investigation focused on determining the role Gas 6 plays in endothelial cell survival and in Axl-related apoptotic cell death (FF 2, 6). The Examiner has not adequately explained why an ordinary artisan studying the effects of Gas 6 HPAEC on Axl-mediated apoptosis of HPAECs, as taught by Healy, would have been prompted to assay the expression of αvβ3, an angiogenesis marker, in those cells. Moreover, the

Examiner has not pointed to any evidence suggesting that an ordinary artisan would have considered αvβ3 expression relevant, or even useful, in studying Gas 6 or Axl in the manner described in Healy.

The fact that αvβ3 expression *might* have provided *some* useful information regarding Healy's HPAEC cells is, in our view, insufficient to support a conclusion of prima facie obviousness. *See Procter & Gamble v. Teva,* 566 F.3d at 997 ("[P]atents are not barred just because it was obvious 'to explore a new technology or general approach that seemed to be a promising field of experimentation, where the prior art gave only general guidance as to the particular form of the claimed invention or how to achieve it.'")(quoting *In re O'Farrell*, 853 F.2d. at 903).

Accordingly, we reverse the Examiner's obviousness rejection of claim 12.

Claims 15-18 read as follows:

15. The method of claim 1, wherein the compound is an antibody.

16. The method of claim 1, wherein the compound is an antisense molecule.

17. The method of claim 1, wherein the compound is an RNAi molecule.

18. The method of claim 1, wherein the compound is a small organic molecule.

Claims 41-44 read essentially identically to claims 15-18, except that they depend from claim 27. Claims 57-60 also read essentially identically to claims 15-18, except that they depend from claim 56.

We acknowledge the suggestions in Panzer, Ruoslahti, and Klinghoffer that antibodies, antisense molecules, interfering RNA

molecules, and small organic molecules are useful as test compounds in inhibition assays (FF 10-16).

However, as noted above, Healy's study focused on specific molecules, Gas 6, Axl, and Rse, and their interactions and effects on HPAECs (FF 1-6). Thus, we are not persuaded that an ordinary artisan performing such studies would have had a reason to substitute antibodies, antisense molecules, interfering RNA molecules, or other small organic molecules, for the Gas 6 molecule studied in Healy's Axl phosphorylase and cell-based assays. Accordingly, we reverse the Examiner's obviousness rejection of claims 15-18, 41-44, and 57-60.

SUMMARY

We affirm the Examiner's rejection of claims 1, 14, 27, 54-56, and 61 under 35 U.S.C. § 102(b) as anticipated by Healy.

However, we reverse the Examiner's rejection of claims 12, 15-18, 41-44, and 57-60 under 35 U.S.C. § 103(a) as being obvious over Healy, Varner, Ruoslahti, Panzer, and Klinghoffer.

TIME PERIOD

No time period for taking any subsequent action in connection with this appeal may be extended under 37 C.F.R. § 1.136(a).

AFFIRMED-IN-PART

dm

KLARQUIST SPARKMAN, LLP
121 SW SALMON ST
SUITE 1600
PORTLAND, OR 97204

American Journal of Pathology, Vol. 154, No. 4, April 1999

Expression of Receptor Tyrosine Kinase Axl and its Ligand Gas6 in Rheumatoid Arthritis

Evidence for a Novel Endothelial Cell Survival Pathway

Kristy O'Donnell, Inge Clara Harkes, Loretta Dougherty, and Ian Peter Wicks

From the Reid Rheumatology Laboratory, Division of Autoimmune Diseases and Transplantation, The Walter and Eliza Hall Institute of Medical Research, Post Office Royal Melbourne Hospital, Victoria, Australia

Angiogenesis and synovial cell hyperplasia are characteristic features of rheumatoid arthritis (RA). Many growth and survival factors use receptors belonging to the tyrosine kinase family that share conserved motifs within the intracellular catalytic domains. To understand further the molecular basis of cellular hyperplasia in RA, we have used degenerate primers based on these motifs and RNA obtained from the synovium of a patient with RA to perform reverse transcriptase-polymerase chain reaction. We report detection of the receptor tyrosine kinase (RTK) Axl in RA synovium and we document the expression pattern of Axl in capillary endothelium, in vascular smooth muscle cells of arterioles and veins, and in a subset of synovial cells in RA synovial tissue. Gas6 (for growth arrest-specific gene 6), which is a ligand for Axl and is related to the coagulation factor protein S, was found in synovial fluid and tissue from patients with RA and osteoarthritis. Axl expression and function was studied in human umbilical vein endothelial cells (HUVECs). Gas6 bound to HUVECs; soluble Axl inhibited this binding. Exogenous Gas6 protected HUVECs from apoptosis in response to growth factor withdrawal and from TNFα-mediated cytotoxicity. These findings may reveal a new aspect of vascular physiology, which may also be relevant to formation and maintenance of the abnormal vasculature in the rheumatoid synovium. *(Am J Pathol 1999, 154:1171–1180)*

Rheumatoid arthritis (RA) is characterized by hyperplasia of synovial cells, angiogenesis, and a chronic inflammatory cell infiltrate.[1] Although most attention has focused on the infiltration and activation of leukocytes in the synovial compartment in RA, increasing evidence suggests an important contribution from the resident cells, including endothelial cells and synovial cells.[2] Angiogenesis is a characteristic feature of synovial inflammation in RA and adhesion of inflammatory cells to the endothelium is central to the maintenance of tissue inflammation.[3,4]

A number of receptor tyrosine kinase (RTK)-ligand interactions have been identified that regulate vascular development and angiogenesis.[5] Gene knockout mice have been particularly informative in understanding the role of RTKs in the developmental biology of the vascular system. Vascular endothelial cell growth factor (VEGF) is one of the key regulators of vascular development and has two RTK receptors, VEGFR-1 and -2. Mice lacking VEGFR-2 die early in embryonic development due to lack of endothelial and hemopoietic cells,[6] whereas VEGFR-1-null mice generate both these cell types, but die because of failure to form early vascular structures.[7] Another RTK involved in vascular development is Tie-2. Tie-2-null mice generate endothelial cells and early vascular patterning, but cannot organize an appropriate lattice of supporting cells to stabilize the developing vascular network.[8] With the exception of physiological processes such as those in the female reproductive cycle and wound healing, angiogenesis is usually a pathological process in the adult.[9] VEGF is probably the major regulator of angiogenesis in the adult, but basic fibroblast growth factor, platelet-derived growth factor, and hepatocyte growth factor signaling through cognate RTKs, as well as a variety of inflammatory cytokines and their receptors, can also cause angiogenesis.[3,4] Endothelial cells are normally long-lived, but much less is known about survival signals in this cell type, particularly in the presence of tissue inflammation.

Cell growth and survival are active and interconnected processes that depend on the integration of signals from the external environment and the intrinsic differentiation programs of particular cells.[10] In the absence of appropriate signals, cells die by apoptosis. Many growth factor receptors belong to the RTK family. RTKs have unique extracellular domains that specifically bind growth factor

Supported by the Reid Charitable Trusts and the Australian National Health and Medical Research Council.

Accepted for publication January 22, 1999.

Address reprint requests to Professor Ian Wicks, Head, Reid Rheumatology Laboratory, Division of Autoimmune Diseases and Transplantation, The Walter and Eliza Hall Institute of Medical Research, Post Office Royal Melbourne Hospital, Victoria 3050. Australia. E-mail: wicks@wehi.edu.au.

ligands but share homologous intracellular kinase domains that have intrinsic kinase activity and also bind signal transduction molecules. The presence of conserved motifs within the catalytic domain of RTKs has been exploited in the search for new members of the TK family.[11]

As one approach to understanding the molecular basis of cellular hyperplasia in RA, we used reverse transcriptase-polymerase chain reaction (RT-PCR) to search for RTKs expressed in RA synovium. We report the identification of the RTK Axl in RA synovium and the expression of Axl in endothelial cells and vascular smooth muscle cells. We have also found Gas6, a recently discovered ligand for Axl, in synovial fluid. Exogenous Gas6 bound to human umbilical vein endothelial cells (HUVECs) and protected these cells from apoptosis in response to growth factor withdrawal and also from TNFα-mediated cytotoxicity. These findings may reveal a novel survival pathway for endothelial cells, which may be relevant to the pathology of RA.

Materials and Methods

Patients

Synovial fluid samples were obtained from three RA patients, one ballet dancer with a traumatic knee effusion, and one patient with psoriatic arthritis. Synovial tissue samples were obtained from eight patients with RA and six with osteoarthritis (OA) at the time of joint replacement surgery.

Cloning of Axl via RT-PCR from RA Synovial Tissue

A cloning technique based on RT-PCR using degenerate oligonucleotides was used to search for RTKs in RA synovium. RNA was extracted from the synovium of a patient with RA as described below and used to generate cDNA using a cDNA synthesis kit (Amersham, Buckinghamshire, UK). Primers corresponding to sequence motifs within the catalytic domains of protein tyrosine kinase (PTK) family members were used as described elsewhere.[12] The PCR products obtained were gel purified and digested with *Bam*HI and *Eco*RI before ligation to *Bam*HI/*Eco*RI-digested pBluescript II plasmid DNA (Stratagene, La Jolla, CA). Following transformation, plasmid DNA was isolated from individual bacterial colonies and the sequence of the insert was determined using the PCR oligonucleotide primers, a *Taq* Dye Deoxy Terminator Cycle Sequencing Kit (Perkin Elmer, Norwalk, CT) and an ABI automated sequencer (Perkin Elmer).

RNA Analysis

Total RNA was extracted from synovium using guanidinium thiocyanate and electrophoresed in agarose containing formaldehyde. Poly A-+ mRNA was extracted from SV40-transformed synovial cells (SV40.SYN)[13] using oligo(dT)-cellulose. Northern blots were performed by capillary transfer to nylon membranes (Hybond N+, Amersham) and hybridized to ^{32}P-labeled, full length Axl cDNA (Mega Prime DNA labeling system, Amersham). Axl cDNA was provided by Dr. Johannes Janssen.

Immunohistochemistry

Synovial tissue specimens from patients with RA were fixed in paraformaldehyde and embedded in paraffin. Paraffin sections 5 μm thick were dewaxed, hydrated, and incubated in methanol containing 3% peroxidase. Sections were digested with pepsin for 10 minutes at 37°C (Digest-All Kit, Zymed Laboratories, San Francisco, CA), then washed in phosphate-buffered saline (PBS). Immunohistochemistry was performed using a streptavidin-horseradish peroxidase system with 3-amino-9-ethylcarbazole as the chromogen, according to the manufacturer's instructions (Histostain-Plus Kit, Zymed Laboratories). The primary antibody (anti-Axl) was diluted 1:20 in PBS and incubated on the sections overnight at 4°C in a humidified chamber. As a negative control, normal rabbit IgG (Sigma Chemical Co., Steinheim, Germany) was substituted for anti-Axl at a corresponding protein concentration. The sections were counterstained with hematoxylin and mounted (DAKO, Glostrup, Denmark).

Protein Analysis

Protein was extracted from synovial tissue specimens using 25 mmol/L Tris, pH 7.5, 150 mmol/L NaCl, 0.1% TritonX-100, and protease inhibitors (10 μmol/L E-64, 100 μmol/L leupeptin, 10 mmol/L EDTA, 1 μmol/L pepstatin, 1 mmol/L phenylmethylsulfonyl fluoride, 10 mmol/L 1,10-phenanthroline, and 10 μmol/L Z-Phe-Ala-CHN_2, all from Sigma). Synovial tissue lysates were ultracentrifuged at 50,000 rpm for 1 hour at 4°C and frozen at −80°C. Synovial fluids were centrifuged at 2000 rpm for 10 minutes at 4°C to remove cellular components and treated with hyaluronidase at 37°C for 1 hour. For primary cells and cell lines, protein was extracted using 25 mmol/L Tris, pH 7.5, 150 mmol/L NaCl, and 1% 3–3-cholamidopropyl-dimethylamonnio-1-propanesulfonate containing protease inhibitors as above. Lysates were incubated on ice for 30 minutes and cell debris was removed by centrifugation in a microfuge at 13,000 rpm for 10 minutes at 4°C. The amount of protein was estimated using the Bio-Rad Protein Assay (Bio-Rad, Richmond, CA). Samples (75 μg of synovial fluid or 25 μg of synovial tissue lysate) were run on 7.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels and transferred to nitrocellulose (Hybond-C, Amersham). Membranes were blocked with 10% skim milk powder in Tris-buffered saline, 0.1% Tween 20 (TBS-T) and incubated with primary antibody diluted 1:1000 in 1% skim milk powder in TBS-T. In some experiments, Axl and Gas6 anti-sera were preincubated for 30 minutes at room temperature with 5 μg of the extracellular domain of Axl fused to human immunoglobulin (Axl-Ig) or recombinant human Gas6 (rhGas6)

as specificity controls. After washing in TBS-T, blots were incubated with sheep anti-rabbit horseradish peroxidase (Silenus, Hawthorn, Australia) diluted in 1% skim milk powder in TBS-T and proteins were visualized using the ECL detection system (Amersham). Gas6, anti-Gas6 rabbit polyclonal antibody, and Axl-Ig peptide were provided by Dr. Brian Varnum and anti-Axl rabbit polyclonal antibody was provided by Dr. Edison Liu.

Cell Culture Conditions

For *in vitro* culture of synovial lining cells, synovial tissue from RA patients was dissected and minced into 2–3 mm pieces. The tissue was washed in RPMI and dissociated for 1.5 hours at 37°C with gentle agitation in RPMI containing 2.4 mg/ml dispase II (Boehringer Mannheim, Mannheim, Germany), 1 mg/ml collagenase Type II (Sigma), and 100μg/ml DNase I (Boehringer Mannheim) in RPMI. The tissue was then ground gently through a sieve and washed several times in RPMI containing 10% fetal bovine serum (FBS, Life Technologies, Auckland, New Zealand). Cells were cultured in RPMI containing 10% FBS at 37°C, 5% CO_2, with medium changes at 24 hours to remove nonadhering cells and debris. Early passage HUVECs were cultured at 37°C in 5% CO_2 in complete medium consisting of M199 (Earle's salts) medium for endothelial cells supplemented with 25% (v/v) conditioned medium, 20% (v/v) FBS, 50 ng/ml transferrin (Boehringer Mannheim), 10 ng/ml endothelial cell growth supplement (Sigma), 10 μg/ml insulin (Sigma), and 2 mmol/L glutamine (Life Technologies, Grand Island, NY). The cells were seeded in 24-well plates precoated in 2.5% (w/v) gelatin (BDH Chemicals, Poole, UK) in PBS at approximately 5×10^5 cells per well.

Gas6 Binding to HUVECs

HUVECs were detached using trypsin/EDTA and incubated in complete M199 medium either with or without 500 ng/ml Gas6 for 1 hour at 37°C. In some experiments, 10 μg/ml soluble Axl-Ig was added in addition to Gas6. After washing in PBS containing 2% FBS, the cells were stained with anti-Gas6 polyclonal antibody at 10 μg/ml or an irrelevant rabbit antibody, washed, and stained with fluorescein isothiocyanate-labeled sheep anti-rabbit Ig (Silenus). Stained cells were analyzed on a Becton Dickinson (San Jose, CA) FACScan.

Cell Survival Assay

Cells were grown in complete medium (containing serum and growth factors), M199 medium (to induce cell death by apoptosis), or M199 medium supplemented with 100 ng/ml Gas6. The medium was changed every 48 hours and cell death was monitored at days 1, 2, 5, and 8 after detachment of the cells using trypsin/EDTA. Viable cells were counted using trypan blue exclusion. There were three replicates for each time point and the experiment was performed three times.

TNFα-Mediated Cytotoxicity

Cells were grown in M199 base medium supplemented with 0, 10, or 100 ng/ml Gas6. At day 1, 10^{-8} mol/L TNFα (Boehringer Ingelheim, Frankfurt, Germany) was added to half the wells without changing the medium to induce cell death by apoptosis. Fresh Gas6 was added at day 2 and cells were harvested by trypsinization at day 5. Viable cells were counted using trypan blue exclusion. There were four replicates for each condition and the experiment was performed three times.

Cell Cycle Analysis by Flow Cytometry

HUVECs collected from a TNFα-mediated cytotoxicity experiment were washed in PBS, fixed in 70% ethanol, and stained with propidium iodide as described previously.[14] Measurement of propidium iodide fluorescence and analysis of the cell cycle was performed on a Becton Dickinson FACScan using CellFit SOBR computer software. The combination of dead and apoptotic cells was measured by counting the percentage of events to the immediate left of the G1 histogram peak. Ten thousand events were collected for each sample.

Statistical Analysis

Student's *t*-test was used to measure the difference between group means.

Results

Cloning of Axl from Rheumatoid Synovial Tissue Using RT-PCR for Tyrosine Kinases

To detect members of the PTK family we performed RT-PCR using degenerate oligonucleotide primers that correspond to sequences within the catalytic domain of PTK family members. RNA was extracted from the synovium of a patient with RA and, after synthesis of cDNA, tyrosine kinase sequences were amplified using PTK-I and PTK-II oligonucleotides as primers.[12] A PCR product of approximately 200 bp was obtained, purified, and subcloned into pBluescript II. After transformation of competent *E. coli*, individual bacterial colonies were picked and plasmid DNA isolated for sequence determination. Members of the jak family of tyrosine kinases were the clones most frequently obtained, but DNA sequences from multiple distinct colonies revealed 100% homology with the RTK Axl,[15] also known as UFO.[16] We chose to study the expression and possible function of this RTK in RA in more detail.

Axl Expression in RA Synovium

Northern Blotting

Northern blot analysis was performed to assess the level of Axl expression in synovial tissues. Human Axl mRNA occurs as two transcripts of 4.9 and 3.4 kb. These

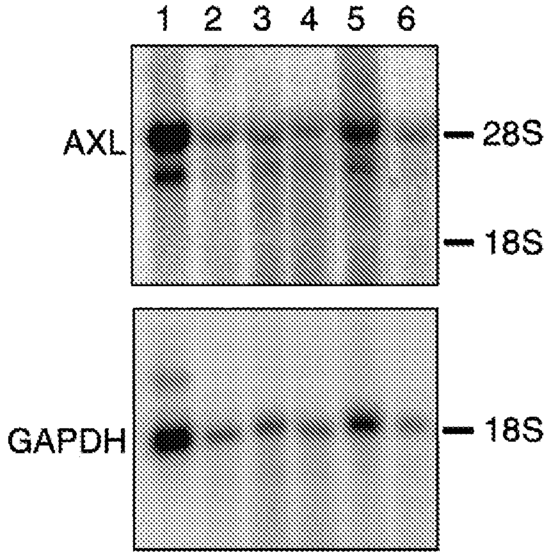


Figure 1. Northern blot analysis of Axl mRNA expression in synovial tissues. PolyA+ mRNA was extracted from an SV40-transformed synovial cell line, SV40.SYN (lane 1), and total mRNA from synovium of patients with RA (lanes 2–5) and OA (lane 6). cDNA containing the entire Axl coding sequence was used as the probe (upper panel). The same Northern blot was subsequently probed with glyceraldehyde-3-phosphate dehydrogenase cDNA to control for RNA loading (lower panel).

gene products are generated by alternative splicing of exon 10 and differential usage of two imperfect polyadenylation sites.[17] Figure 1 shows Axl mRNA in synovial tissues from several patients with RA and one patient with OA. OA was used as a control for rheumatoid joint disease. Both mRNAs are present in all samples, as well as in the SV40.SYN cell line, showing that Axl expression is not unique to RA.

Immunohistochemistry

We next performed immunohistochemistry to determine which cell types express Axl within RA synovial tissue (Figure 2). The most striking finding was of Axl expression associated with blood vessels, in particular endothelial cells. In subsynovial capillaries, Axl was expressed in endothelial cells (Figure 2, D and E). However, in larger blood vessels, both arterioles and veins, Axl expression was confined to smooth muscle cells (Figure 2, B and C). Expression was also seen in some but not all synovial lining cells in RA synovium (Figure 2F). Normal rabbit IgG used at the same protein concentration as the Axl polyclonal antibody gave widespread nonspecific staining (Figure 2A).

Western Blotting

To confirm our immunohistochemistry findings, protein lysates from a number of relevant cell types were analyzed for Axl expression by Western blotting (Figure 3A). Axl is a 140-kd glycosylated protein.[14] Soluble Axl-Ig was used a positive control (molecular weight, 110 kd). Primary cultured RA synovial cells showed low level, but detectable Axl expression and this was greater in the synovial cell line SV40.SYN. However, in accord with the immunohistochemistry results, HUVEC protein lysates were strongly positive for Axl. A number of lower molecular weight immunoreactive bands were also detected in HUVECs, consistent with multiple glycosylation sites in the extracellular domain of Axl.[18] The specificity of the anti-Axl antibody was shown by pre-incubation with the soluble Axl-Ig peptide (Figure 3B) before addition to a Western blot. The 110-kd band of Axl-Ig was almost completely abolished, indicating antibody specificity for the extracellular domain of Axl. Rabbit IgG showed no reactivity against HUVEC lysates or Axl-Ig (data not shown).

Gas6 in Synovial Tissue and Fluid

Gas6 has been identified as a ligand for Axl.[19,20] Protein lysates from synovial tissue of patients with either RA or OA were used in a Western blot to detect expression of Gas6 (Figure 4A). Using a polyclonal anti-Gas6 antibody, a major immunoreactive protein of approximately 75 kd corresponding to recombinant human Gas6 could be identified in all synovial tissue specimens, although the intensity was generally greater in the RA synovial tissues. Equivalent amounts of protein, as estimated by the Bio-Rad protein assay, were loaded in each lane. Specificity of the anti-Gas6 antibody was confirmed by competition with recombinant human Gas6 (Figure 4B). Figure 4C shows Western blot analysis of synovial fluids from patients with RA, psoriatic arthritis, and a noninflammatory joint effusion probed with the anti-Gas6 antibody. A band of approximately 75 kd was identified in all synovial fluid specimens, corresponding to the expected size of Gas6. A second band of approximately 90 kd was also seen, possibly corresponding to a previously described Gas6 splice variant.[21] The proteolytic products of this variant are thought to be approximately 36 and 50 kd,[22] and immunoreactive bands of this size were identified in the Western blot of synovial fluid (Figure 4C) but not synovial tissue.

Gas6 Binds to HUVECs

Gas6 has been shown to be a ligand for Axl and the related RTKs Sky and Mer.[19,23–25] To confirm that Gas6 is a physiological ligand for Axl expressed by HUVECs, Gas6 was added to HUVECs and the cells were stained with an anti-Gas6 antibody. As shown in Figure 5, HUVECs bound added Gas6 and this was competed out in the presence of soluble Axl-Ig.

Gas6 Prolongs Survival of Endothelial Cells Following Growth Factor Withdrawal

Gas6 is able to protect a variety of cells from apoptosis induced by complete growth factor depletion.[26–28] To determine whether Gas6 has similar activity in HUVECs, we induced apoptosis by complete growth factor depletion (Figure 6). In the growth factor-deprived cultures, cell

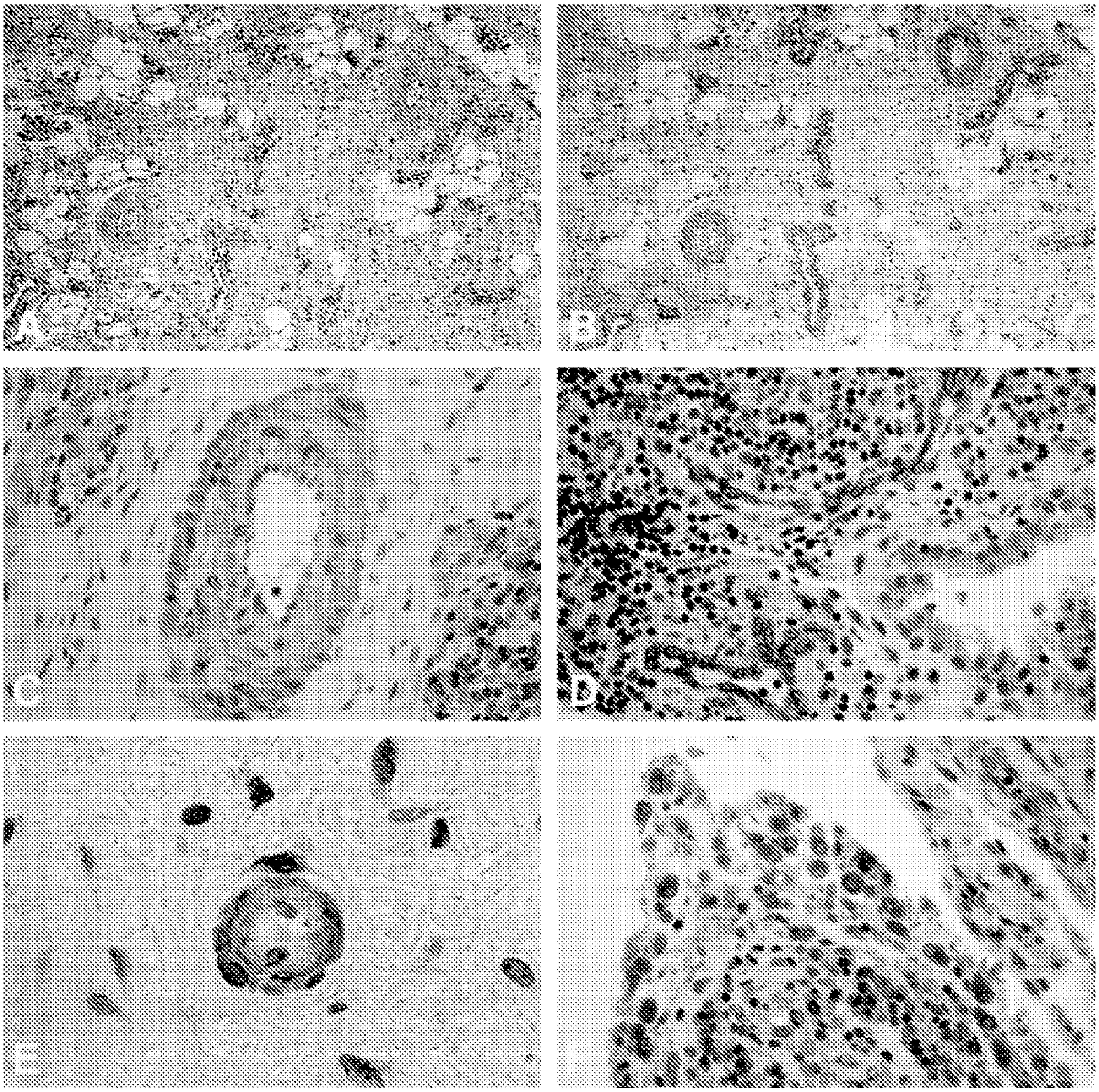


Figure 2. Immunohistochemistry of Axl expression in RA synovium. **A**: Immunoreactivity with an equivalent concentration of normal rabbit IgG. **B-F**: Immunoreactivity with rabbit anti-Axl polyclonal antibody (staining for Axl appears as a red precipitate). Axl expression in vascular structures (**B**), in arteriole and capillary (**C**), in subintimal veins (**D**); in capillary (**E**), in synovial lining cells and capillaries (**F**). Final magnification, ×100 (**A** and **B**); ×400 (**C**, **D**, and **F**); ×1000 (**E**).

viability had decreased at 24 hours and by day 8 all cells were dead. In contrast, when Gas6 was added to growth factor-deprived HUVECs, there was an initial drop in viability at 24 hours, but thereafter cell viability was retained. Rescue from apoptosis by Gas6 was statistically significant at days 5 ($P < 0.001$) or 8 ($P < 0.002$). HUVECs produced some endogenous. Gas6 under normal culture conditions (data not shown), but cell-associated Gas6 was clearly unable to rescue HUVECs to the same extent.

Gas6 Protects HUVECs from TNFα-Induced Cell Death

TNFα is known to induce apoptosis of some cell types, especially upon withdrawal of growth factors.[29] Gas6 has been found to rescue TNFα-treated NIH3T3 cells from apoptosis.[27] We therefore examined the ability of Gas6 to protect HUVECs against TNFα-mediated apoptosis. As shown in Figure 7, TNFα efficiently induced cell death in growth factor-starved HUVECs ($P < 0.001$) and 100 ng/ml (but not 10 ng/ml) of Gas6 partially protected HUVECs from TNFα-induced cytotoxicity ($P < 0.001$).

Gas6 Retains HUVECs in the Cell Cycle and Reduces Cell Death

Flow cytometric analysis was used to demonstrate the survival effects of Gas6. Figure 8 shows representative cell cycle profiles of HUVECs under conditions of growth factor deprivation (Figure 8A) and after treatment with

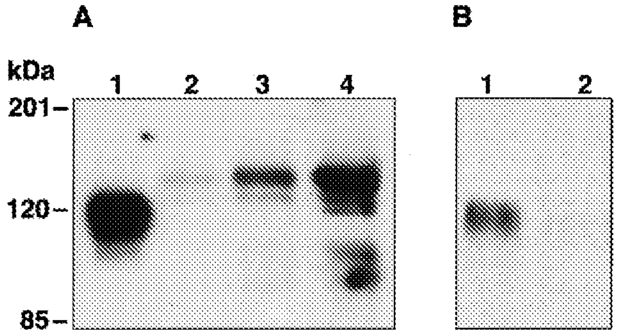


Figure 3. Western blot analysis of Axl in protein extracts from isolated cell types. A: Axl-Ig (lane 1), protein lysates from cultured rheumatoid synovial lining cells (lane 2), an SV40-transformed synovial cell line (SV40.SYN) (lane 3), and HUVECs (lane 4). Equal amounts of protein were loaded in each lane. The Western blot was probed with an anti-Axl rabbit polyclonal antibody. B: Axl-Ig was loaded in lanes 1 and 2. The Western blot was probed with anti-Axl (lane 1) or anti-Axl that had been pre-incubated with Axl-Ig (lane 2).

Gas6 (Figure 8B), TNFα (Figure 8C), or both (Figure 8D). Dead or apoptotic cells accumulate in the hypodiploid region to the left of the vertical marker. Cells retained in the various phases of the cell cycle at the time of sampling appear to the right of the vertical marker. The percentage of apoptotic or dead cells was significantly higher in the growth factor-starved cells compared to starved cells supplemented with Gas6 (65 ± 4% compared with 49 ± 3%, $P < 0.001$). Rescue from TNFα-induced apoptosis by Gas6 was incomplete but statistically significant. This experiment was performed three times with a total of seven replicates for each condition. Because most TNFα-treated cells were killed it was difficult to determine the true hypodiploid region, but there was at least 85 ± 4% cell death with TNFα, compared with 76 ± 6% in the presence of Gas6 ($P < 0.01$). The G1 peak was obliterated in cells treated with TNFα (Figure 8C) but remained obvious when Gas6 was added (Figure 8D), indicating retention of cells in the cell cycle. The percentage of cells in the G1 phase of the cell cycle was significantly higher in the serum-starved, Gas6-treated group compared to cells without Gas6 (20 ± 3% compared with 13 ± 3%, $P < 0.001$).

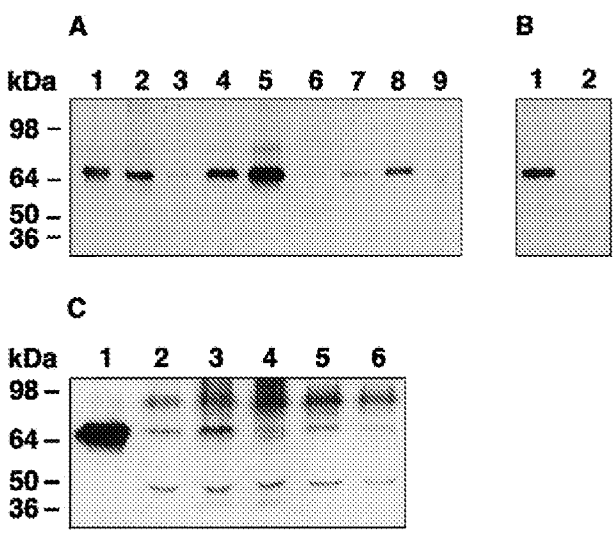


Figure 4. Western blot analysis of Gas6 in synovial tissue and fluid. Equal amounts of protein were loaded in each lane. A: synovial tissue from four separate patients with RA (lanes 2–5) or OA (lanes 6–9). Recombinant human Gas6 (lane 1) was used as a positive control. B: Gas6 probed with anti-Gas6 (lane 1) or with anti-Gas6 antibody pre-incubated with Gas6 (lane 2). C: synovial fluids from three separate patients with RA (lanes 2–4), a traumatic joint effusion (lane 5), and one patient with psoriatic arthritis (lane 6). Gas6 was used as a positive control (lane 1).

Discussion

Resident synoviocytes, endothelial cells, and chondrocytes undergo dramatic changes in response to chronic joint inflammation in diseases such as RA. Understanding the activation, proliferation, survival, and apoptotic pathways of these cells is crucial to understanding this disease. Tyrosine kinases are closely associated with the regulation of growth, survival, and signaling in a wide variety of cells. To study the molecular basis of cellular hyperplasia in RA, we have used RT-PCR to search for RTKs expressed in rheumatoid synovial tissue. This approach has been used by a number of investigators to characterize TKs that are overexpressed by tumors[30] but has not been previously used to study the pathology of RA. We identified Axl, an RTK originally discovered through its association with chronic myeloid leukemia.[15,16] Using immunohistochemistry, we found Axl expressed by some synovial cells, but the most striking expression was associated with vascular structures, in particular with smooth muscle cells and endothelial cells. Endothelial cell expression of Axl has not been previously noted and so we decided to investigate potential functional effects of Axl and its ligand Gas6 in this cell type, using HUVECs as a model.

Axl expression has been found in myeloid, erythroid, and megakaryocytic leukemic cell lines,[31] in myeloid leukemias,[32] and in colonic[33] and hepatocellular carcinomas.[30] ARK (the murine counterpart of Axl) is expressed within mesenchymal elements by day 12.5 of murine embryonic development[34] and is broadly expressed in adult mouse tissues.[16] Less is known about the cellular distribution of Axl, but rat vascular smooth muscle cells,[35,36] human chondrocytes,[37] human CD34+ hemopoietic stem cells, and mature myeloid hemopoietic cells[32] have been shown to express Axl. Our study is the first to show clear expression of Axl by human endothelial cells and vascular smooth muscle cells *in situ* and provides further support for the possibility that Axl may be involved in vascular structure or function. We found an intriguing pattern of Axl expression: in small capillaries, Axl was expressed by endothelial cells, whereas in larger arterioles and veins, surrounding smooth muscle cells were Axl-positive. The extracellular domain of Axl contains adjacent fibronectin type III and immunoglobulin-like repeats[16,38] and homophilic binding between the extracellular domains of Axl has been demonstrated.[39] This suggests a role in cell adhesion which could be relevant to tube formation in angiogenesis. Vascular smooth muscle cell expression has been previously noted in the rat and may suggest involvement of Axl in some other aspect of vascular function.[35,36] Clearly, the

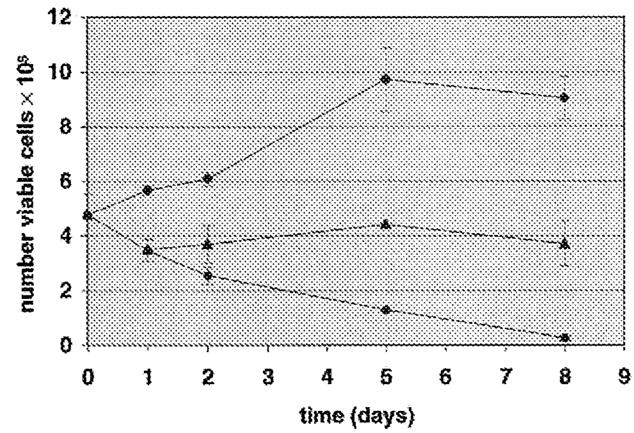


Figure 6. Effect of Gas6 on growth factor-deprived HUVECs. HUVECs were grown in complete medium (**diamonds**) or induced to undergo apoptosis by withdrawal of growth factors in the presence (**triangles**) or absence (**circles**) of 100ng/ml Gas6. Cell viability was assessed at indicated time points by trypan blue exclusion. Results are presented as the mean ± SD of data collected from three replicates in a representative experiment. The experiment was performed three times.

phenotype of mice with a targeted deletion of Axl will be of great interest in this regard, both in the basal state and in response to inflammatory and angiogenic stimuli.

One ligand for Axl has been identified as Gas6.[19,20] Gas6 was originally discovered and named due to its production by cells in the quiescent phase of the cell cycle.[40] Gas6 is a multimodular protein with an N-terminal γ-carboxyglutamic acid (Gla) domain, epidermal growth factor-like repeats, and a sex hormone-binding globulin-like domain.[41] The last feature may be sufficient for receptor binding and activation.[42,43] Gas6 requires vitamin K-dependent γ-carboxylation and has homology to Protein S, a key protease regulator of coagulation.[41] The full spectrum of Gas6 biological activity is currently under investigation, but it is of interest that Protein S and several other serum proteases including thrombin,[44] urokinase-type plasminogen activator,[45] and factor Xa[46] have also been found to contribute to inflammatory pathways.

Gas6 has a number of properties that may be relevant to vascular biology. Gas6 expression has been documented in unstimulated endothelial cells [41,47] and conditioned media from a bovine endothelial cell line was used to stimulate Axl phosphorylation and subsequently to purify Gas6 as an Axl ligand.[20] Gas6 was also found in conditioned media of rat vascular smooth muscle cells that had been treated with thrombin and endothelin.[48] Gas6 can promote adhesion between Axl-expressing cells[49] and can elicit chemotaxis of vascular smooth muscle cells.[50] Both of these properties are reminiscent of the Tie-2 ligand angiopoietin-1 and Gas6 could be similarly involved in formation or modeling of the vasculature. Avanzi et al[47] reported that Gas6 inhibited adhe-

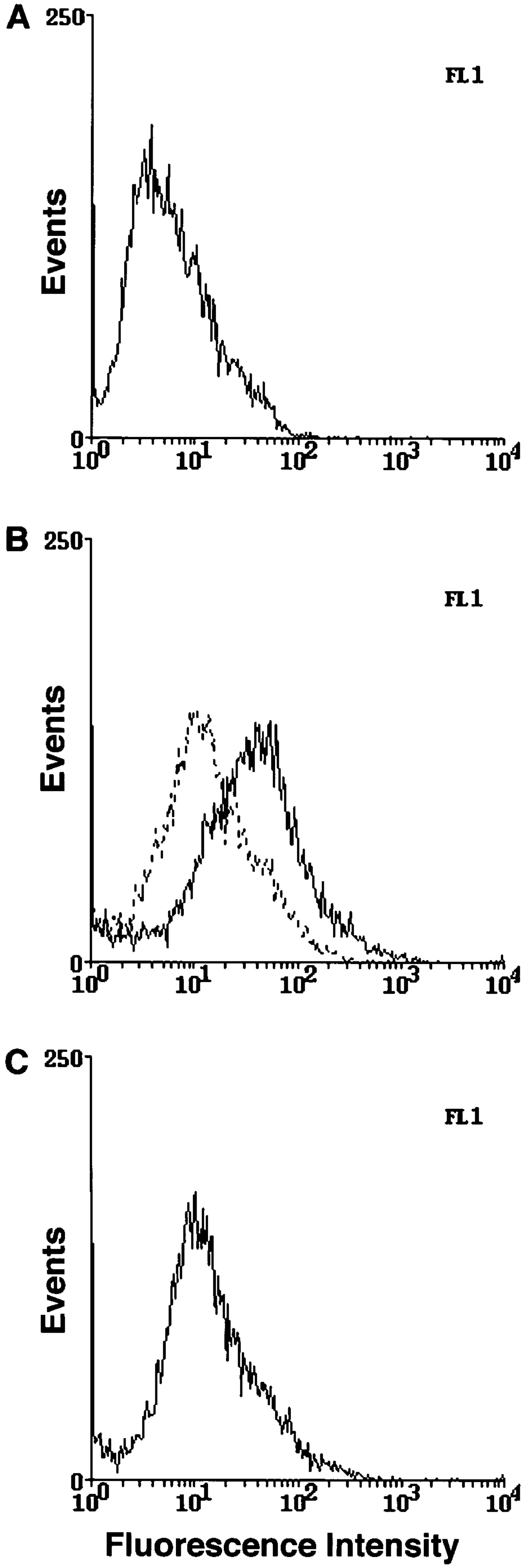


Figure 5. Detection of Gas6 binding to HUVECs by flow cytometry. **A**: HUVECs stained with an irrelevant rabbit polyclonal antibody (at an equivalent concentration to the test antibody). **B**: HUVECs stained with an anti-Gas6 polyclonal antibody either with (filled line) or without (dotted line) the addition of Gas6 to the cells for 1 hour. **C**: addition of Gas6 and soluble Axl to HUVECs to compete out Gas6 binding.

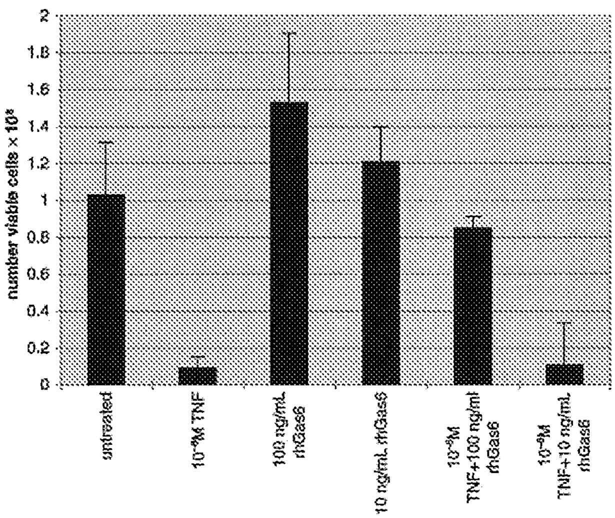


Figure 7. HUVECs in growth factor-free conditions were treated with TNFα, both with and without Gas6. Results are presented as mean ± SD of four replicates from a representative experiment. The experiment was performed three times.

sion of neutrophils to stimulated, but not resting, HUVECs and speculated that Gas6 exerts a protective anti-inflammatory effect. Nakano et al[51] showed that the Gla domain of Gas6 can specifically bind phosphatidylserine, a phospholipid normally positioned on the inner leaflet of the plasma membrane but thought to be exposed on dying cells, leading those investigators to propose a role for Gas6 in the clearance of apoptotic cells.

We found Gas6 in synovial tissue and fluid from patients with OA and RA. Endothelial cells,[41,47] rat vascular smooth muscle cells,[48] and cultured human chondrocytes[37] have been found to produce Gas6 and these cell types are therefore potential sources of Gas6 in synovial fluid. However, to our knowledge Gas6 has not been detected in the serum, suggesting local production or an alteration of half-life within the joint. It is of interest that the levels of Gas6 were generally higher in RA synovial tissue, suggesting that Gas6 may be up-regulated or overproduced in the setting of joint inflammation.

Gas6 is now well characterized as a promiscuous ligand for the Axl subfamily but, in contrast to most RTK ligands, Axl-Gas6 interaction alone induces only modest mitogenic effects in some cells.[26,35,37,48,52–54] However, Gas6 has been shown to protect a number of Axl-positive cells from stimuli that induce apoptosis.[26–28,37] Other nonmitogenic properties of Gas6 include chemotactic effects on vascular smooth muscle cells[50] and up-regulation of osteoclast function.[55] A number of effects of Gas6 on vascular smooth muscle cells have been documented;[28,35,48,50] however, much less is known about Axl-Gas6 interaction in endothelial cells. We chose HUVECs as a model system and have shown that these cells express Axl and bind Gas6. Upon growth factor withdrawal, exogenous Gas6 acted as a survival factor for HUVECs and protected them from TNFα-induced cytotoxicity. Little is known about regulation of endothelial cell survival and how it changes in inflammation.[56] The synovial cavity is a relatively hypoxic and acidotic envi-

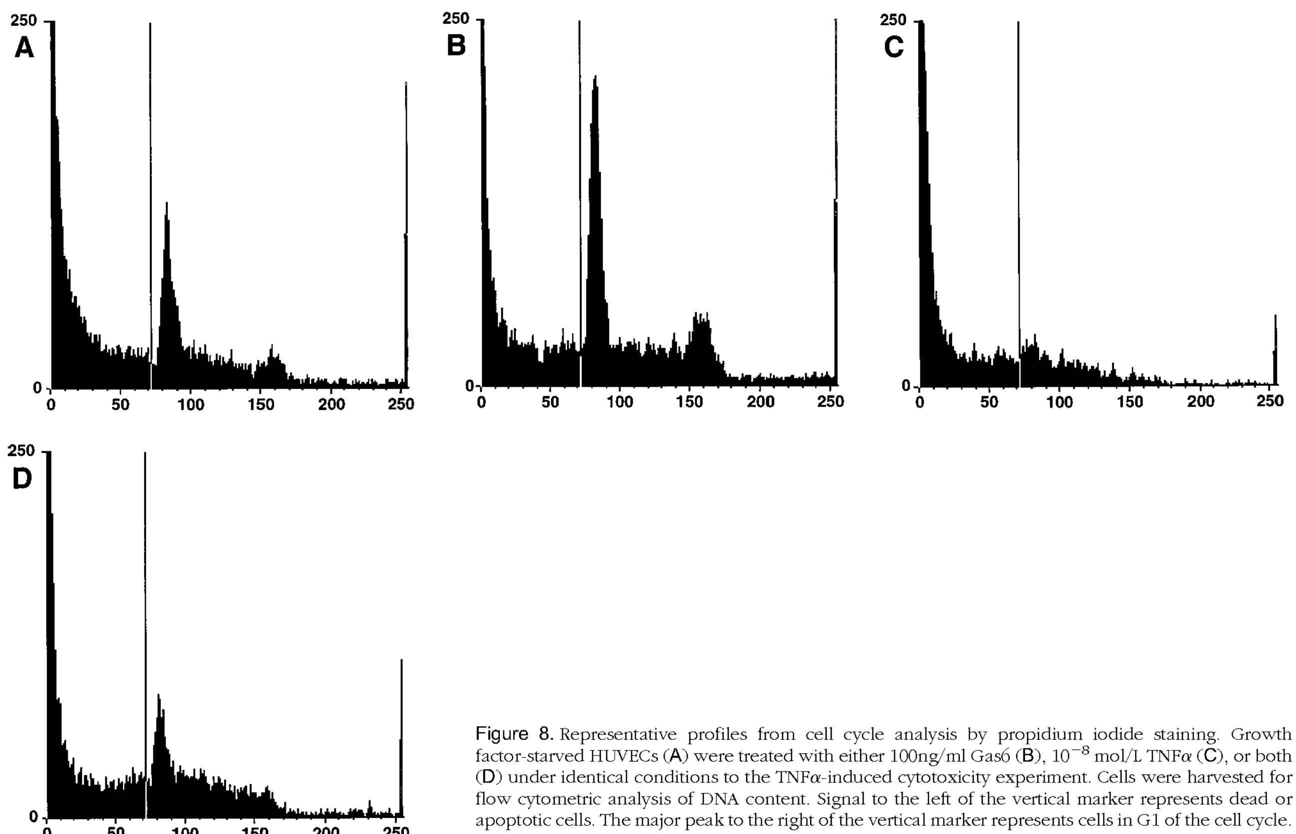


Figure 8. Representative profiles from cell cycle analysis by propidium iodide staining. Growth factor-starved HUVECs (A) were treated with either 100ng/ml Gas6 (B), 10^{-8} mol/L TNFα (C), or both (D) under identical conditions to the TNFα-induced cytotoxicity experiment. Cells were harvested for flow cytometric analysis of DNA content. Signal to the left of the vertical marker represents dead or apoptotic cells. The major peak to the right of the vertical marker represents cells in G1 of the cell cycle. The experiment was performed three times.

ronment and synovial effusions can result in ischemia of the synovium.[57] Inflammatory cytokines such as TNF are produced in high local concentrations in RA and attract leukocytes from the bloodstream. The major role of Axl-Gas6 interaction may therefore be in survival of the vasculature under conditions of cellular stress or injury.[36]

Within the normal synovial joint, Axl and Gas6 could function as a survival pathway for endothelial cells and perhaps for vascular smooth muscle cells, synovial cells, and chondrocytes. Our results raise the possibility that Gas6 may also promote survival of activated endothelial cells, and perhaps other Axl-positive cells, within the hostile environment of the inflamed rheumatoid joint. In this way, a survival mechanism normally involved in tissue homeostasis could also contribute to maintenance of a pathological vasculature in RA.

Acknowledgments

We gratefully acknowledge the support of the Reid Charitable Trusts and the Australian National Health and Medical Research Council. We also thank Drs. Geoff McColl and Kaku Nakagawa for generously providing some of the synovial tissue and synovial fluid samples. We thank Drs. Brian Varnum, Edison Liu, and Johannes Janssen for their kind gifts of reagents. We thank Dr. Gino Vairo for assistance with the flow cytometric analysis and Dr. Henry De Aizpurua for helpful comments on the manuscript.

References

1. Harris ED: Rheumatoid arthritis: pathophysiology and implications for therapy. N Engl J Med 1990, 322:1277–1289
2. Firestein GS: Invasive fibroblast-like synoviocytes in rheumatoid arthritis: passive responders or transformed aggressors? Arth Rheum 1996, 11:1781–1790
3. Koch AE: Angiogenesis-implications for rheumatoid arthritis. Arth Rheum 1998, 41:951–962
4. Jackson JR, Seed MP, Kricher CH, Willoughby DA, Winkler JD: The codependence of angiogenesis and chronic inflammation. FASEB J 1997, 11:457–465
5. Hanahan D: Signalling vascular morphogenesis and maintenance. Science 1997, 277:48–50
6. Shalaby F, Rossant J, Yamaguchi TP, Gertsenstein M, Wu XF, Breitman ML, Schuh AC: Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice. Nature 1995, 376:62–66
7. Fong GH, Rossant J, Gertsenstein M, Breitman ML: Role of the Flt-1 receptor tyrosine kinase in regulating the assembly of the vascular endothelium. Nature 1995, 376:66–70
8. Sato TN, Tozawa Y, Deutsch U, Wolburg-Buchholz K, Fujiwara Y, Gendron-Maguire M, Gridley T, Wolburg H, Risau W, Qin Y: Distinct roles of the receptor tyrosine kinases Tie-1 and Tie-2 in blood vessel formation. Nature 1995, 376:70–74
9. Folkman J: Angiogenesis in cancer, vascular, rheumatoid and other disease. Nat Med 1995, 1:27–30
10. Raff M: Social controls on cell survival and cell death. Nature 1992, 356:397–400
11. Hanks SK, Lindberg RA: Use of degenerate oligonucleotide probes to identify clones that encode protein kinases. Methods Enzymol 1991, 200:525–532
12. Wilks AF: Two putative protein-tyrosine kinases identified by application of the polymerase chain reaction. Proc Natl Acad Sci USA 1989, 86:1603–1607
13. Wicks IP, Leizer T, Wawryk SO, Novotny JR, Hamilton J, Vitti G, Boyd AW: The effect of cytokines on the expression of MHC antigens and ICAM-1 by normal and transformed synoviocytes. Autoimmunity 1992, 12:13–20
14. Vadiveloo PK, Vairo G, Novak U, Royston AK, Whitty G, Filonzi EL, Cragoe Jr EJ, Hamilton J: Differential regulation of cell cycle machinery by various antiproliferative agents is linked to macrophage arrest at distinct G1 checkpoints. Oncogene 1996, 13:599–608
15. O'Bryan JP, Frye RA, Cogswell PC, Neubauer A, Kitch B, Prokop C, Espinosa R, Le Beau MM, Earp HS, Liu ET: Axl, a transforming gene isolated from primary human myeloid leukaemia cells, encodes a novel receptor tyrosine kinase: Mol Cell Biol 1991, 11:5016–5031
16. Janssen JWG, Schulz AS, Steenvoorden ACM, Schmidberger M, Strehl S, Ambros PF, Bartram CR: A novel putative tyrosine kinase receptor with oncogenic potential. Oncogene 1991, 6:2113–2120
17. Schulz AS, Schleithoff L, Faust M, Bartram CR, Janssen JWG: The genomic structure of the human UFO receptor. Oncogene 1993, 8:509–513
18. O'Bryan JP, Fridell Y, Koski R, Varnum B, Liu ET: The transforming receptor tyrosine kinase, Axl, is post-translationally regulated by proteolytic cleavage. J Biol Chem 1995, 270:551–557
19. Varnum B, Young C, Elliott G, Garcia A, Bartley TD, Fridell YW, Hunt RW, Trail G, Clogston C, Tosco RJ, Yanagihara D, Bennett L, Sylber M, Merewether LA, Tseng A, Escobar E, Liu ET, Yamane H: Axl receptor tyrosine kinase stimulated by the vitamin K-dependent protein encoded by growth-arrest-specific gene 6. Nature 1995, 373: 623–626
20. Stitt TN, Conn G, Gore M, Lai C, Bruno J, Radziejewski C, Mattson K, Fisher J, Gies DR, Jones PF, Masiakowski P, Ryan TE, Tobkes NJ, Chen DH, DiStefano PS, Long GL, Basilico C, Goldfarb MP, Lemke G, Glass DJ, Yancopoulos GD: The anticoagulation factor protein S and its relative, Gas 6, are ligands for the Tyro 3/Axl family of receptor tyrosine kinases. Cell 1995, 80:661–670
21. Marcandalli P, Gostissa M, Varnum B, Goruppi S, and Schneider C: Identification and tissue expression of a splice variant for the growth arrest-specific gene gas6. FEBS Lett 1997, 415:56–58
22. Goruppi S, Yamane H, Marcandalli P, Garcia A, Clogston C, Gostissa M, Varnum B, Schneider C: The product of a gas6 splice variant allows the release of the domain responsible for Axl tyrosine kinase receptor activation. FEBS Lett 1997, 415:59–63
23. Godowski PJ, Mark MR, Chen J, Sadick MD, Raab H, Hammonds RG: Re-evaluation of the roles of Protein S and Gas6 as ligands for the receptor tyrosine kinase Rse/Tyro 3. Cell 1995, 82:355–358
24. Chen J, Carey K, Godowski PJ: Identification of Gas6 as a ligand for Mer, a neural cell adhesion molecule related receptor tyrosine kinase implicated in cellular transformation. Oncogene 1997, 14:2033–2039
25. Nagata K, Ohashi K, Nakano T, Arita H, Zong C, Hanafusa H, Mizuno K: Identification of the product of growth arrest-specific gene 6 as a common ligand for Axl, Sky, and Mer receptor tyrosine kinases. J Biol Chem 1996, 271:30022–30027
26. Goruppi S, Ruaro E, Schneider C: Gas 6, the ligand of Axl tyrosine kinase receptor, has mitogenic, and survival activities for serum starved NIH3T3 fibroblasts. Oncogene 1996, 12:471–480
27. Bellosta P, Zhang Q, Goff S, Basilico C: Signaling through the ARK tyrosine kinase receptor protects from apoptosis in the absence of growth stimulation. Oncogene 1997, 15:2387–2397
28. Nakano T, Kawamoto K, Hiogashino K, Arita H: Prevention of growth arrest-induced cell death of vascular smooth muscle cells by a product of growth arrest-specific gene, Gas6. FEBS Lett 1996, 387:78–80
29. Chinnaiyan AM, Tepper CG, Seldin MF, O'Rourke K, Kischkel FC, Hellbardt S, Krammer PH, Peter ME, Dixit VM: FADD/MORT1 is a common mediator of CD95 (Fas/APO-1), and tumor necrosis factor receptor-induced apoptosis. J Biol Chem 1996, 271:4961–4965
30. Tsou AP, Wu KM, Tsen TY, Chi CW, Chiu JH, Lui WY, Hu CP, Chang C, Chou CK, Tsai SH: Parallel hybridisation analysis of multiple protein kinase genes: identification of gene expression patterns characteristic of human hepatocellular carcinoma. Genomics 1998, 50:331–340
31. Challier C, Uphoff CC, Janssen JWG, Drexler HG: Differential expression of the ufo/Axl oncogene in human leukemia-lymphoma cell lines. Leukaemia 1996, 10:781–787
32. Neubauer A, Fiebeler A, Graham DK, O'Bryan JP, Schmidt CA, Barckow P, Serke S, Siegert W, Snodgrass HR, Huhn D, Liu ET: Expression of Axl, a transforming receptor tyrosine kinase, in normal and malignant hematopoiesis. Blood 1994, 84:1931–1941

33. Craven RJ, Xu LH, Weiner TM, Fridell YW, Dent GA, Srivasta S, Varnum B, Liu ET, Cance WG: Receptor tyrosine kinases expressed in metastatic colon cancer. Int J Cancer 1995, 60:791–797
34. Faust M, Ebensperger C, Schulz AS, Schleithoff L, Hameister H, Bartram CR, Janssen JWG: The murine UFO receptor: molecular cloning, chromosomal localization and in situ expression analysis. Oncogene 1992, 7:1287–1293
35. Nakano T, Kawamoto K, Kishino J, Nomura K, Higashino K, Arita H: Requirement of γ-carboxyglutamic acid residues for the biological activity of Gas6: contribution of endogenous Gas6 to the proliferation of vascular smooth muscle cells. Biochem J 1997, 323:387–392
36. Melaragno MG, Wuthrich D, Poppa V, Gill D, Lindner V, Berk B, Corson M: Increased expression of Axl tyrosine kinase after vascular injury and regulation by G-protein coupled receptor agonists in rats. Circ Res 1998, 83:697–704
37. Loeser RF, Varnum BC, Carlson CS, Goldring MB, Liu ET, Sadiev S, Kute TE, Wallin R: Human chondrocyte expression of growth-arrest-specific gene 6 and the tyrosine kinase receptor Axl. Arth Rheum 1997, 40:1455–1465
38. Rescigno J, Mansukhani A, Basilico C: A putative receptor tyrosine kinase with unique structural topology. Oncogene 1991, 6:1909–1913
39. Bellosta P, Costa M, Lin DA, Basilico C: The receptor tyrosine kinase ARK mediates cell aggregation by homophilic binding. Mol Cell Biol 1995, 15:614–625
40. Schneider C, King RM, Philipson L: Genes specifically expressed at growth arrest of mammalian cells. Cell 1988, 54:787–793
41. Manfioletti G, Brancolini C, Avanzi G, Schneider C: The protein encoded by a growth arrest-specific gene (Gas6) is a new member of the vitamin K-dependent proteins related to protein S, a negative coregulator in the blood coagulation cascade. Mol Cell Biol 1993, 13:4976–4985
42. Mark MR, Chen J, Hammonds RG, Sadick M, Godowski PJ: Characterization of Gas6, a member of the superfamily of G domain-containing proteins, as a ligand for Rse and Axl. J Biol Chem 1996, 271: 9785–9789
43. Tanabe K, Nagata K, Ohashi K, Nakano T, Arita H, Mizuno K: Roles of γ-carboxylation and a sex hormone-binding globulin-like domain in receptor-binding and in biological activities of Gas6. FEBS Lett 1997, 408:306–310
44. Shin H, Nakajima T, Kitajima I, Shigeta K, Abeyama K, Imamura T, Okano T, Kawahara K, Nakamura T, Marumaya I: Thrombin receptor-mediated synovial proliferation in patients with rheumatoid arthritis. Clin Immunol Immunopathol 1995, 76:225–233
45. Lansink M, Koolwijk P, van Hinsbergh V, Kooistra T: Effect of hormones and retinoids on the formation of capillary-like tubular structures of human microvascular endothelial cells in fibrin matrices is related to urokinase expression. Blood 1998, 92:927–938
46. Cirino G, Cicala C, Bucci M, Sorrentino L, Ambrosini G, DeDominicis G, Altieri DC: Factor Xa as an interface between coagulation and inflammation. J Clin Invest 1997, 99:2446–2451
47. Avanzi GC, Gallicchio M, Bottarel F, Gammaitoni L, Cavalloni G, Buonfiglio D, Bragardo M, Bellomo G, Albano E, Fantozzi R, Garbarino G, Varnum B, Aglietta M, Saglio G, Dianzani U, Dianzani C: Gas6 inhibits granulocyte adhesion to endothelial cells. Blood 1998, 91:2334–2340
48. Nakano T, Higashino K, Kikuchi N, Kishino J, Nomura K, Fujita H, Ohara O, Arita H: Vascular smooth muscle cell-derived, Gla-containing growth-potentiating factor for Ca^{2+}-mobilising growth factors. J Biol Chem 1995, 270:5701–5705
49. McCloskey P, Fridell YW, Attar E, Villa J, Jin Y, Varnum B, Liu E: Gas6 mediates adhesion of cells expressing the receptor tyrosine kinase Axl: J Biol Chem 1997, 272:23285–23291
50. Fridell YW, Villa J, Attar EC, Liu ET: Gas6 induces Axl-mediated chemotaxis of vascular smooth muscle cells. J Biol Chem 1998, 273:7123–7126
51. Nakano T, Ishimoto Y, Kishino J, Umeda M, Inoue K, Nagata K, Ohashi K, Mizuno K, Arita H: Cell adhesion to phosphatidylserine mediated by a product of growth arrest-specific gene 6. J Biol Chem 1997, 272:29411–29414
52. Goruppi S, Ruaro E, Varnum B, Schneider C: Requirement of phosphatidylinositol 3-kinase-dependent pathway and Src for Gas6-Axl mitogenic and survival activities in NIH3T3 fibroblasts. Mol Cell Biol 1997, 17:4442–4453
53. Li RH, Chen J, Hammonds G, Phillips H, Armanini M, Wood P, Bunge R, Godowski PJ, Sliwkowski MX, Mather JP: Identification of Gas6 as a growth factor for human Schwann cells. J Neurosci 1996, 16:2012–2019
54. Tanaka K, Nagayama Y, Nakano T, Takamura N, Namba H, Fukada S, Kuma K, Yamashita S, Niwa M: Expression profile of receptor-type protein tyrosine kinase genes in the human thyroid. Endocrinol 1998, 139:852–858
55. Nakamura YK, Hakeda Y, Takakura N, Kameda T, Hamaguchi I, Miyamoto T, Kakudo S, Nakano T, Kumegawa M, Suda T: Tyro 3 receptor tyrosine kinase, and its ligand, Gas6, stimulate the function of osteoclasts. Stem Cells 1998, 16:229–238
56. Bach FH, Hancock WH, Ferran C: Protective genes expressed in endothelial cells: a regulatory response to injury. Immunol Today 1997, 18:483–486
57. Mapp PI, Grootveld MC, Blake DR: Hypoxia, oxidative stress, and rheumatoid arthritis. Br Med Bull 1995, 51:419–436